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PARTHENOGENESE UND POLYPLOIDIE BEI RÜSSELKÄFERN (CURCULIONIDAE)

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(With a Summary in English)

OBWOHL die parthenogenetische Vermehrung bei den Käfern im allgemeinen sehr selten ist, machen die Rüsselkäfer, Curculionidae, in dieser Hinsicht eine Ausnahme. Bei ihnen ist nämlich die Parthenogenese in den Unterfamilien Otiorrhynchinae und Brachyderinae eine verhältnismässig allgemeine Erscheinung. Es ist festgestellt worden (SUOMALAINEN, 1940 a und b), dass die meisten parthenogenetischen Curculionidenarten polyploid sind. Von den vom Verf. (op. c.) untersuchten finnischen Arten erwies sich nur eine (*Polydrosus mollis*) als diploid mit 22 Chromosomen. Fünf Arten (*Otiorrhynchus ovatus*, *O. ligustici*, *Strophosomus melanogrammus*, *Trachyphloeus bifoveolatus* und *Sciaphilus asperatus*) sind triploid mit 33 oder einer anderen nahestehenden Zahl von Chromosomen. Drei Arten (*Otiorrhynchus dubius*, *O. scaber* und *Barynotus obscurus*) wiederum haben 44 Chromosomen und sind dadurch tetraploid. Weil die Polyploidie im Tierreich sehr selten ist, verdient diese bei den parthenogenetischen Rüsselkäfern festgestellte polyploide Artenreihe in mancher Hinsicht ein gewisses Interesse.

Um die Parthenogenese und Polyploidie bei den Rüsselkäfern möglichst gut kennen zu lernen und zu verstehen, ist es notwendig, möglichst viele Arten zytologisch zu untersuchen, und zwar sogar in verschiedenen Teilen ihrer betreffenden Verbreitungsgebiete. Man erhält dadurch u. a. die Möglichkeit, einerseits die eventuell vorhandenen fortpflanzungsbiologisch und zytologisch verschiedenen Rassen ein und derselben Art — bzw. entsprechende einander nahestehende Arten — zu finden und ihre Verbreitung kennen zu lernen, anderseits auch die Chromosomenverhältnisse einander entsprechender bisexueller und parthenogenetischer Rassen bzw. Arten miteinander zu vergleichen.

Im folgenden werden die Chromosomenverhältnisse einiger mitteleuropäischen und bisher nicht untersuchten finnischen Curculionidenarten beschrieben. Die meisten hier behandelten Arten gehören der

umfangreichen Gattung *Otiorrhynchus* an, einer Gattung, die gute Möglichkeiten zur Klärung einiger wesentlichen Fragen der Parthenogenese bietet, weil in ihrem Bereich viele einander nahestehende bisexuelle wie auch parthenogenetische Formen anzutreffen sind (Näheres bei APFELBECK, 1928 und 1930; SZÉKESSY, 1937; SUOMALAINEN, 1940 b, S. 21—26; JAHN, 1941).

I. MATERIAL UND METHODEN.

Die vorliegende Arbeit betrifft die Chromosomenverhältnisse bei insgesamt 20 Curculionidenarten. Von diesen fallen die meisten der Gattung *Otiorrhynchus* zu. Die untersuchten *Otiorrhynchus*-Arten sind die folgenden (System und Nomenklatur nach WINKLER [1932]; die von mir früher [op. c.] zytologisch untersuchten Arten sind in Klammern beigegeben):

	Bisexuell		Parthenogenet.
	Spermatogen.	Oogenese	Oogenese
Untergattung *Dodecastichus* STIERL.			
**O. inflatus* GYLL.	+		
O. geniculatus GERM.	+	+	
Untergattung *Otiorrhynchus* s. str.			
O. sensitivus SCOP.	+	+	
**O. armadillo* ROSSI	+	+	
O. bisulcatus F.	+	+	
O. niger F.	+	+	+
O. fuscipes OL.	+		
O. morio F.	+	+	
(*O. arcticus* O. F.)	(+)	(+)	
(*O. dubius* STRÖM)			(+)
**O. scaber* L.			+(+)
Untergattung *Dorymerus* SEIDL.			
O. austriacus F.		+	
**O. equestris* RICHT.	+		
O. singularis L.			+
**O. pupillatus* GYLL.			+
O. salicis STRÖM	+	+	+
O. sulcatus F.			+
O. gemmatus SCOP.	+	+	+
Untergattung *Tournieria* STIERL.			
(*O. ovatus* L.)			(+)
Untergattung *Arammichnus* GOZIS			
(*O. ligustici* L.)			(+)
Untergattung *Tyloderes* SCHÖNH.			
O. chrysops HBST.		+	

Neben den obenangeführten *Otiorrhynchus*-Arten werden die Chromosomenverhältnisse noch bei drei anderen parthenogenetischen Rüsselkäferarten behandelt. Diese sind *Sciaphilus asperatus* BONSD., *Strophosomus melanogrammus* FÖRST. und **Barynotus moerens* F. Die mit einem Sternchen (*) bezeichneten Arten hat Herr Professor Dr. K. HOLDHAUS (Wien) bestimmt oder meine Bestimmung bestätigt, wofür ich ihm bestens danke.

Das Untersuchungsmaterial stammt hauptsächlich aus der Umgebung der Biologischen Station Lunz (Lunz am See) in den Österreichischen Kalkalpen. Ein kleinerer Teil desselben ist in der Umgebung von Berlin oder in Helsinki gesammelt worden. Genauere Angaben über den Ursprung und den Umfang des Materials sind im Zusammenhang mit den Artenbesprechungen zu finden.

Zur Untersuchung der Spermatogenese wurden Testes von Imagines in 0,6-prozentiger Normosallösung herauspräpariert. Zur Fixierung und Färbung ist Karminessigsäure verwendet worden (Quetsch-Dauerpräparate nach dem bei BAUER und TIMOFÉEFF-RESSOVSKY [1939] angegebenen Verfahren). Häufig wurden die Präparate mit dem Gemisch von BOUIN-ALLEN (BAUERS Modifikation; Näheres bei BAUER, 1931) nachfixiert und mit fuchsinschwefliger Säure nach FEULGEN nachgefärbt. Wenn mit dem Nukleolus zusammenhängende Fragen zu untersuchen waren, wurde auch Methylgrün oder Eisenhämatoxylin (nach HEIDENHAIN) zur Nachfärbung verwendet.

Die Ovarien und die abgelegten Eier wurden mit CARNOYs Gemisch (6 : 3 : 1) fixiert, weil dieses in die Eier gut und schnell eindringt, und danach über Chloroform in Parovax eingebettet. Sämtliche abgelegten Eier und die schwerer schneidbaren Ovarien wurden jedoch nach der Buthylalkohol-Methode in das Parovax gebracht. Die Dicke der Parovaxschnitte betrug bei den Ovarien sowie bei den abgelegten Eiern 15 μ. Sie wurden nach FEULGEN gefärbt.

Die Zeichnungen sind mit Hilfe des Objektivs 120 $\times$ (Apochromat), des Okulars 25 $\times$ und des ABBEschen Zeichenapparats (alles Zeiss) auf der Höhe des Objekttisches ausgeführt worden. Die Vergrösserung ist, nachdem die Abbildungen beim Klischieren auf $^2/_3$ der ursprünglichen Grösse verkleinert sind, also 2000fach. In einigen Abbildungen wurden die Chromosomen zur besseren Übersicht getrennt gezeichnet oder umgeordnet; das ist in den betreffenden Bildertexten mit dem Zeichen $\longleftrightarrow$ vermerkt.

In den Bildertexten gelten folgenden Abkürzungen: KES = Fixierung und Färbung mit Karminessigsäure. KES + B—A + Feulg. =

= Fixierung und Färbung mit Karminessigsäure; Nachfixierung mit dem Gemisch von BOUIN-ALLEN und Nachfärbung nach FEULGEN. C + Feulg. = Fixierung mit CARNOYS Gemisch und Färbung nach FEULGEN.

II. DIE CHROMOSOMENVERHÄLTNISSE BEI DEN UNTERSUCHTEN CURCULIONIDEN.

1. DIE BISEXUELLEN ARTEN UND RASSEN.

Wie aus dem auf S. 426 Gesagten hervorgeht, betrifft die vorliegende Arbeit die Chromosomenverhältnisse bei insgesamt 13 bisexuellen *Otiorrhynchus*-Arten. Die Spermatogenese ist bei 11 Arten und die Oogenese bei 10 Arten untersucht worden. Die untersuchten Individuen verteilen sich auf die verschiedenen Arten wie folgt: *O. inflatus* 1 ♂; *O. geniculatus* 2 ♂♂, 1 ♀; *O. sensitivus* 3 ♂♂, 3 ♀♀, *O. armadillo* 1 ♂, 1 ♀; *O. bisulcatus* 1 ♂, 1 ♀; *O. niger* 2 ♂♂, 5 ♀♀ (dazu wurden 5 parthenogenetische ♀♀ untersucht); *O. fuscipes* 2 ♂♂; *O. morio* 2 ♂♂, 4 ♀♀; *O. austriacus* 1 ♀; *O. equestris* 2 ♂♂; *O. salicis* 2 ♂♂, 1 ♀ (dazu 3 parthenogenetische ♀♀ untersucht); *O. gemmatus* 3 ♂♂, 1 ♀ (dazu 4 parthenogenetische ♀♀ untersucht); *O. chrysops* 4 ♀♀. Dieses Material stammt in seiner Gänze aus Lunz am See.

Weil die untersuchten bisexuellen *Otiorrhynchus*-Arten alle dieselbe Chromosomenzahl haben und weil sie auch in bezug auf die Meiose einander ähneln, werden sie im folgenden gemeinsam behandelt. Die Grösse der Chromosomen variiert jedoch einigermassen von Art zu Art.

A. DIE SPERMATOGENESE.

Die diploide Chromosomenzahl des Männchens beträgt bei sämtlichen untersuchten Arten 22; dieselbe diploide Chromosomenzahl habe ich auch bei den von mir früher untersuchten bisexuellen Curculionidenarten festgestellt (vgl. SUOMALAINEN, 1940 a und b). Zwei von den Chromosomen sind Geschlechtschromosomen. Das Geschlechtschromosomenpaar besteht beim *Otiorrhynchus*-Männchen aus einem *X*- und einem sehr kleinen *Y*-Chromosom.

Spermatogonienchromosomen. — Es ist mir gelungen Spermatogonienpräparate nur von wenigen Arten zu erhalten. In Fig. 1 ist eine Metaphaseplatte bei *Otiorrhynchus fuscipes* abgebildet. Die meisten von den 20 Autosomen, vielleicht alle, haben ein nahezu medianes Zentromer. Ein Chromosom, das wahrscheinlich das *X*-Chromosom ist, scheint telozentrisch zu sein. Das *Y* ist rundlich und recht klein.

Die *früheren Stadien der Meiose* verlaufen nach dem gewöhnlichen Schema. Im Leptotän sieht man im Kern lange, dünne Chromosomenfäden, die unregelmässig gewunden sind und einen dichten Knäuel bilden. Im späten Leptotän und im frühen Pachytän kann man eine

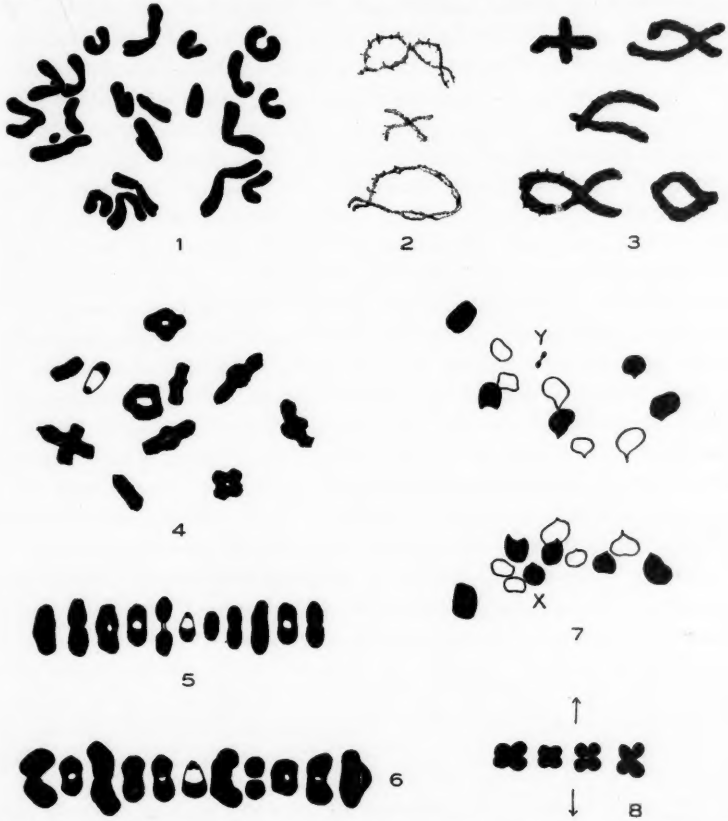

Fig. 1. *Otiorrhynchus fuscipes.* Fig. 2—3 und 6—8. *O. sensitivus.* Fig. 4. *O. morio.* Fig. 5. *O. salicis.* — Fig. 1. Spermatogonienmetaphase. — Fig. 2. Diplotäntetraden beim ♂. — Fig. 3. Tetraden der frühen Diakinese beim ♂. — Fig. 4. Diakinese beim ♂. — Fig. 5—6. Profilbilder der 1. Metaphase beim ♂ (⟷). — Fig. 7. Profilbild der 1. Anaphase beim ♂. — Fig. 8. Profilbild der 2. Metaphase beim ♂ (nicht alle Chromosomen sind eingezeichnet). — Fig. 1 und 5—7. KES. Fig. 2—4 und 8. KES + B—A + Feulg.

recht deutliche Bukettorientierung der Chromosomen wahrnehmen. Weil ich kein genau analysierbares Zygotänstadium gefunden habe,

lässt es sich nicht ermitteln, wie die Konjugation im einzelnen stattfindet. Im späteren Pachytän verschwindet die Bukettorientierung, und die Chromosomen, die jetzt ihrer ganzen Länge nach eng konjugiert sind, liegen nun zerstreut im Kernraum. Im Pachytän sieht man in allen Kernen einen verhältnismässig grossen Nukleolus, der deutlich dem heteropyknotischen *X*-Chromosom angeheftet ist. Auch das *Y*-Chromosom scheint mit dem Nukleolus zusammenzuhängen.

Tetradengenese. — Nach dem Pachytän trennen sich die konjugierten homologen Chromosomen schnell voneinander, womit das Diplotänstadium beginnt. Ein besonderes Strepsitänstadium kann bei keiner Art wahrgenommen werden. Im Diplotän und in der Diakinese gewahrt man bei sämtlichen Arten typische und deutliche Chiasmata. Sowohl deren Symmetrischwerden als deren Terminalisation finden auf allgemein bekannte Weise statt. Im Diplotän und in den Anfangsstadien der Diakinese (Fig. 2—3) ist die Kontraktion der Chromosomen noch gering, sodass dieselben lang und schlank erscheinen. Die Chiasmata sind nur in wenigen Fällen symmetrisch geworden und ihre Terminalisation befindet sich erst im Anfang. Die meisten Tetraden haben deutlich nur ein Chiasma; doch sieht man auch Tetraden mit zwei Chiasmata. Ein paarmal habe ich auch solche Tetraden gefunden, bei denen anscheinend drei Chiasmata vorkommen (vgl. Fig. 2). Die dritte als ein Chiasma erscheinende Stelle in ihnen kann natürlich auch eine durch Torsion der Partner herbeigeführte optische Überkreuzung sein.

In der späteren Diakinese (Fig. 4) ist die Kontraktion deutlich fortgeschritten, sodass die Chromosomen kürzer und dicker sind. Die Chiasmata sind im allgemeinen schon symmetrisch und oft auch vollständig terminalisiert. Die meisten Tetraden sind typische Stabtetraden; man sieht jedoch auch Kreuztetraden, sogar solche mit verhältnismässig langen Querbalken. Neben den erwähnten Tetradentypen findet man auch typische Ringtetraden mit zwei an verschiedenen Enden terminalisierten Chiasmata. Die Zahl der Ringtetraden in einer Zelle ist bei verschiedenen Arten ein wenig verschieden, wie der folgende Vergleich zwischen *O. sensitivus* und *O. morio* (gezählt an 50 Spermatozyten) zeigt:

	Zellen ohne Ringtetr.	Zellen mit 1 Ringtetr.	Zellen mit 2 Ringtetr.	Zellen mit 3 Ringtetr.	Zellen mit 4 Ringtetr.	Zellen mit 5 Ringtetr.	Zellen mit 6 Ringtetr.
O. sensitivus	15	18	11	6	—	—	—
O. morio	2	4	12	13	12	6	1

Bei *O. sensitivus* schwankt demgemäss die Zahl der Ringtetraden in einer Zelle zwischen 0 und 3 und bei *O. morio* zwischen 0 und 6. Bei

der ersteren Art findet man in einem Kern am häufigsten also einen Ring, bei *O. morio* aber 3 solche.

Reifungsteilungen. — In der 1. Metaphase gewahrt man 10 Autosomentetraden und das *XY*-Paar (Fig. 5—6). Die Lage der Tetraden wird durch die Koorientierung der beiden homologen Zentromeren bestimmt. Die Längsachsen der Tetraden sind nämlich parallel zur Spindel eingestellt. Die Kontraktion der Chromosomen ist jetzt maximal und die Chiasmata sind mit wenigen Ausnahmen völlig terminalisiert. In den meisten Metaphaseplatten findet man auch einige Ringtetraden mit zwei terminalisierten Chiasmata. Die zusammenhängenden *X*- und *Y*-Chromosomen sind nach verschiedenen Polen gerichtet.

In der 1. Anaphase (Fig. 7) trennen sich die konjugierten Chromosomen auf gewöhnliche Weise voneinander. In beiden Platten sieht man 10 Autosomen; dazu kommt in der einen Platte noch das *X*-Chromosom und in der anderen das kleine *Y*-Chromosom. Als Folge der 1. Reifungsteilung entstehen somit zweierlei 2. Spermatozyten, nämlich sowohl solche mit dem *X*- als solche mit dem *Y*-Chromosom. In der 2. Metaphase liegen die Längsachsen der Chromosomen in der Richtung der Äquatorialebene. In Seitenansicht sind die Chromosomen oft kreuzförmig (Fig. 8), weil ihre Chromatiden nur an der Spindelansatzstelle aneinander haften. Bei der 2. Reifungsteilung teilen sich die Chromosomen äquationell.

Der Konjugationsmechanismus der *X*- und *Y*-Chromosomen wird in anderem Zusammenhang näher behandelt.

B. DIE OOGENESE.

Die diploide Chromosomenzahl des Weibchens ist bei allen untersuchten Arten dieselbe wie beim Männchen, nämlich 22. Beim Weibchen sind die beiden Geschlechtschromosomen *X*-Chromosomen.

Oogonienteilungen sind in meinen Präparaten nicht vorhanden. Auch die früheren Stadien der Meiose sowie die Tetradengenese wurden in der Oogenese nicht näher untersucht.

Reifungsteilungen. — In der 1. Metaphase gewahrt man bei allen untersuchten bisexuellen Arten 11 Tetraden, von denen eine das konjugierte *X*-Chromosomenpaar ist (Fig. 9—13). Sie sind meistens sehr regelmässig zur 1. Metaphaseplatte angeordnet. Die meisten Tetraden sind Stabtetraden mit einem terminalisierten Chiasma; nicht selten findet man auch Ringtetraden mit zwei Chiasmata. Wie bei den Spermatozyten, so scheint auch bei den Oozyten die Zahl der Ring-

tetraden in einer Zelle bei verschiedenen Arten ein wenig verschieden zu sein. Eine Untersuchung der Oozyten von *O. sensitivus* und *O. morio* gab nämlich in dieser Hinsicht folgendes Resultat.

	Zellen ohne Ringtetr.	Zellen mit 1 Ringtetr.	Zellen mit 2 Ringtetr.	Zellen mit 3 Ringtetr.	Zellen mit 4 Ringtetr.	Zellen mit 5 Ringtetr.
O. sensitivus	2	2	2	—	—	—
O. morio	1(0?)	1(2?)	3	2	—	1

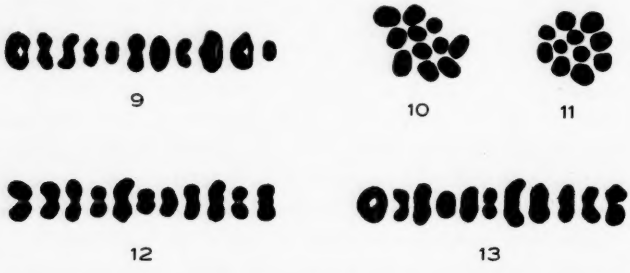

Fig. 9. *Otiorrhynchus morio.* Fig. 10. *O. niger.* Fig. 11—12. *O. salicis.* Fig. 13. *O. gemmatus.* — Fig. 9 und 12—13. Profilbilder der 1. Metaphase beim ♀ der bisexuellen Rasse (←→). — Fig. 10—11. Metaphaseplatten der 1. Reifungsteilung beim ♀ der bisexuellen Rasse. — Alles C + Feulg.

In bezug auf die Zahl der Ringtetraden scheint demgemäss kein grösserer Unterschied zwischen Spermatozyten (vgl. S. 430) und Oozyten zu bestehen. (Das Material ist allerdings nicht gross. Besonders in den Oozyten ist es nämlich nicht leicht die Zahl der Ringtetraden zu bestimmen, weil die Ringe in den Schnittpräparaten teilweise auch im Profil sichtbar sind.)

Die Eier befinden sich bei der Ablage im Metaphasestadium der 1. Teilung. Die beiden Reifungsteilungen erfolgen bald nachdem das Ei abgelegt worden ist. Sie weisen keine Besonderheiten in bezug auf ihren Ablauf auf. In der 2. Teilung teilt sich die innere Platte früher als die äussere, soweit sich die letztgenannte überhaupt teilt.

Bemerkenswert ist der Umstand, dass von drei Arten, nämlich *Otiorrhynchus niger*, *O. salicis* und *O. gemmatus*, in Lunz am See neben diploiden bisexuellen Weibchen auch triploide Weibchen auftreten, die recht wahrscheinlich parthenogenetisch sind.

2. DIE PARTHENOGENETISCHEN ARTEN UND RASSEN.

In der vorliegenden Untersuchung werden die Chromosomenverhältnisse bei insgesamt 10 parthenogenetischen Arten bzw. Rassen be-

handelt. Von diesen sind acht triploid, eine tetraploid und eine pentaploid. Bei sämtlichen machen die Eizellen nur eine Reifungsteilung durch, die eine Äquationsteilung ist.

In der Literatur sind mir keine Angaben über Parthenogenese bei fünf von den untersuchten Arten, nämlich *Otiorrhynchus niger*, *O. singularis*, *O. gemmatus*, *O. pupillatus* und *Barynotus moerens*, begegnet.

A. DIE TRIPLOIDEN ARTEN UND RASSEN.

Otiorrhynchus niger. — Insgesamt zehn Weibchen wurden untersucht. Sie stammen alle aus Lunz am See. Fünf von den Tieren waren diploid mit normaler Chromosomenkonjugation und Tetradenbildung (siehe Fig. 10); sie gehören demnach der bisexuellen Rasse an. Fünf von den untersuchten Weibchen waren dagegen triploid mit 33 Chromosomen. Ihre Eier laufen nur eine Reifungsteilung durch und entwickeln sich darum wahrscheinlich parthenogenetisch. Bei den parthenogenetischen Weibchen kann man in der Metaphaseplatte der Reifungsteilung 33 ungleich grosse univalente Chromosomen finden (Fig. 14). Die exakte Bestimmung der Chromosomenzahl war freilich nur in einer Platte möglich. Platten mit etwas über 30 Chromosomen waren in den Präparaten mehrere enthalten. Eine genauere Analyse der Chromosomentypen ist bei keiner untersuchten Art möglich.

Otiorrhynchus scaber. — Diese Art ist in verschiedenen Gegenden Südfinnlands tetraploid (SUOMALAINEN, 1940 a, S. 58; 1940 b, S. 88—91). Bei den finnischen Exemplaren konnten nämlich in den Metaphaseplatten der Reifungsteilung in den meisten Fällen 44 Chromosomen gezählt werden; man findet jedoch auch Platten mit 42 oder 43 Chromosomen.

Die Art war häufig auf einer ganz beschränkten Fläche in der Nähe der Biologischen Station in Lunz. Sie war dort parthenogenetisch; die gesammelten 130 Exemplare waren alle Weibchen. *Otiorrhynchus scaber* erwies sich in Lunz am See als triploid (vgl. SUOMALAINEN, 1944; 1945, S. 187—188). Insgesamt zehn Weibchen wurden zytologisch untersucht und sie waren alle triploid. In sämtlichen acht Eiern, die eine exakte Bestimmung der Chromosomenzahl erlaubten, wurde diese als 33 gefunden (Fig. 15). Ausserdem wurden in mehreren Metaphaseplatten je etwas über 30 Chromosomen festgestellt. Die triploide Chromosomenzahl wurde auch in Follikelzellen des Ovars konstatiert.

In einem von den untersuchten Ovarien waren in den Prophasekernen weniger als 33 Chromosomenelemente sichtbar. Freilich nur

zwei späte Prophasen wurden gefunden; die eine enthielt 21—22 Chromosomenelemente, die andere nur ungefähr 16. Mehrere von diesen Chromosomenelementen waren bedeutend grösser als die univalenten

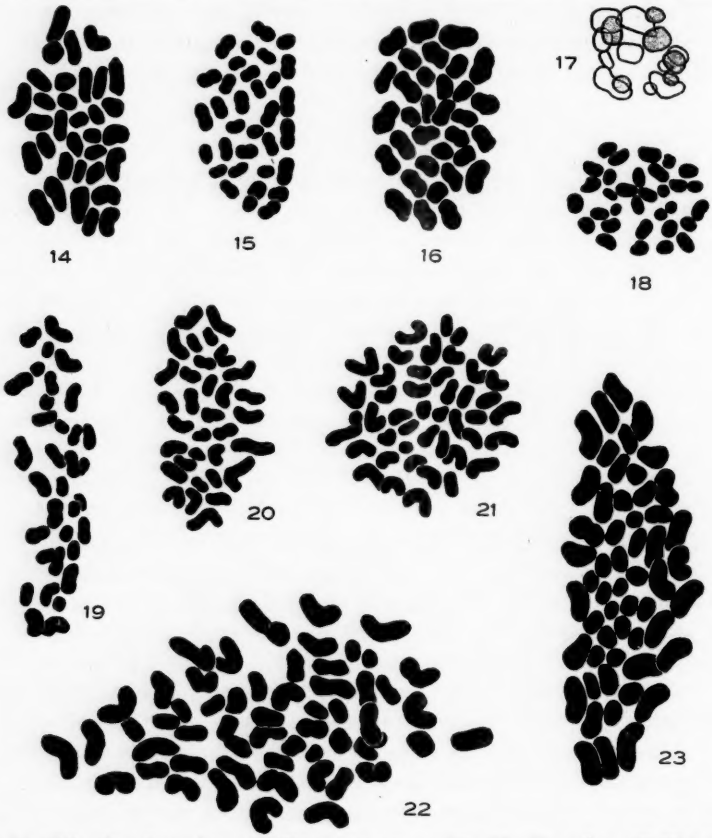

Fig. 14. *Otiorrhynchus niger*. 33 Chromosomen. — Fig. 15. *O. scaber*. 33 Chromosomen. — Fig. 16. *O. singularis*. 33 Chromosomen. — Fig. 17—18. *O. salicis*. 32 Chromosomen. — Fig. 19. *O. sulcatus*. 34 Chromosomen. — Fig. 20. *O. gemmatus*. 33 Chromosomen. — Fig. 21. *O. pupillatus*. 44 Chromosomen. — Fig. 22—23. *Barynotus moerens*. 55 Chromosomen. — Fig. 17. Prophase der Reifungsteilung des parthenogenetischen ♀. Fig. 14—16 und 18—23. Metaphasen der Reifungsteilung des parthenogenetischen ♀. — Alles C + Feulg.

Metaphasechromosomen. Es ist offenbar, dass jene wenigstens zwei Chromosomen enthalten. Diese Frage wird später (S. 435—436) näher diskutiert.

Otiorrhynchus singularis. — Von dieser Art wurden sechs Weibchen zytologisch untersucht. Eines von diesen stammt aus Südfinnland, aus Helsinki; die anderen fünf wurden in Mitteldeutschland, in Berlin-Buch gesammelt. Insgesamt wurden in Berlin-Buch etwa 20 *Otiorrhynchus singularis*-Exemplare erbeutet, und sie alle waren Weibchen. Sämtliche untersuchten Tiere waren triploid und zytologisch gleich. In der Metaphaseplatte der Reifungsteilung sind 33 univalente, oft ein wenig semmelförmige Chromosomen zu finden (Fig. 16). Die exakte Bestimmung der Chromosomenzahl war freilich nur in drei Platten möglich; ausserdem wurden in mehreren Platten ungefähr 33 Chromosomen konstatiert. Die meisten Chromosomen zeigen in ihrer Mitte eine mehr oder weniger deutliche Einschnürung und scheinen daher ein nahezu medianes Zentromer zu haben. (Die nahezu mediane Lage des Zentromers ist auch bei einigen anderen Arten [vgl. die Figuren] bei der Reifungsteilung in den meisten Chromosomen feststellbar.)

Otiorrhynchus salicis. — Vier Weibchen, alle aus Lunz am See, wurden untersucht. Eines von denselben war diploid mit normaler Chromosomenkonjugation und Tetradenbildung (siehe Fig. 11—12). Dieses Weibchen gehört der bisexuellen Rasse an. Drei von den untersuchten Weibchen waren dagegen triploid. Ihre Eier machen nur eine Reifungsteilung durch und entwickeln sich wahrscheinlich parthenogenetisch. Bei den parthenogenetischen Weibchen war die exakte Bestimmung der Chromosomenzahl nur in einer Metaphaseplatte der Reifungsteilung möglich; sie beträgt 32 (Fig. 18). Platten mit etwas über 30 univalenten Chromosomen wurden mehrere gefunden. Bei einem von den untersuchten Weibchen waren die Chromosomen in den Eiern nicht zu einer regelmässigen Metaphaseplatte angeordnet, sondern ganz unregelmässig gruppiert. Die triploide Chromosomenzahl war jedoch auch hier feststellbar.

Bemerkenswert ist der Umstand, dass in den Eiern eines parthenogenetischen *O. salicis*-Weibchens — ganz wie es bei einem *O. scaber*-Weibchen (vgl. S. 434) der Fall war — während der späten Prophase bedeutend weniger als 32—33 Chromosomenelemente sichtbar waren (Fig. 17). Insgesamt wurden über zehn solche Prophasen gefunden. Weil die Chromosomenelemente in der späten Prophase in einer dichten Gruppe stehen, lässt sich ihre Zahl meistens nicht exakt ermitteln. In den fraglichen Prophasen schwankt die Zahl der Chromosomenelemente zwischen etwa 7 und ungefähr 20; meistens beträgt sie etwa 20. Die in Fig. 17 abgebildete Zelle z. B. hat 16 Chromosomenelemente. Die Grössenunterschiede derselben sind viel beträchtlicher als diejenigen der

univalenten Chromosomen in den triploiden Metaphaseplatten. Es ist offenbar, dass die grossen Chromosomenelemente dieser Prophasekerne zwei oder mehrere Chromosomen enthalten. Es ist schwierig mit Sicherheit zu entscheiden, ob die sich zusammenschliessenden Chromosomen homolog sind und an den Chiasmata aneinander haften — also Bivalente oder Trivalente bilden — oder ob zwei oder mehrere Chromosomen sich ohne Chiasmabildung vorübergehend aneinander anschliessen. Der Umstand, dass in entsprechenden Metaphaseplatten nie Bivalente oder Trivalente auftreten und dass in einem Prophasekern die Zahl der Chromosomenelemente sogar weniger als 11 (etwa 7) betrug, spricht für die letztgenannte Alternative.

Otiorrhynchus sulcatus. — Diese in Finnland sonst sehr seltene Art ist häufig auf einer beschränkten Fläche im Botanischen Garten der Universität Helsinki. Sie ist dort parthenogenetisch, denn unter den ungefähr 150 untersuchten Exemplaren wurde kein einziges Männchen gefunden. Insgesamt zehn Weibchen wurden zytologisch untersucht, und sie waren alle triploid. In der Metaphaseplatte der Reifungsteilung kann man bei dieser Art 34 univalente Chromosomen sehen (Fig. 19). Alle fünf Eier, die eine exakte Bestimmung der Chromosomenzahl erlaubten, zeigten in der Metaphaseplatte 34 Chromosomen. Platten mit etwas über 30 Chromosomen waren in den Präparaten reichlich enthalten.

Otiorrhynchus gemmatus. — Fünf Weibchen, alle aus Lunz am See, wurden untersucht. Eines von denselben war diploid mit normaler Chromosomenkonjugation und Tetradenbildung (siehe Fig. 13); es gehört der bisexuellen Rasse an. Vier von den untersuchten Weibchen waren dagegen triploid mit 33 Chromosomen. Weil ihre Eier nur eine Reifungsteilung durchlaufen, entwickeln sie sich offenbar parthenogenetisch. Diese beiden rassenspezifischen Eiertypen wurden auch unter abgelegten Eiern konstatiert. Bei den parthenogenetischen Weibchen kann man in der Metaphaseplatte der Reifungsteilung 33 ungleich grosse univalente Chromosomen finden (Fig. 20). Die exakte Bestimmung der Chromosomenzahl war in fünf Platten möglich. In mehreren Platten wurden etwas über 30 Chromosomen konstatiert.

Sciaphilus asperatus. — Diese Art ist in verschiedenen Gegenden Südfinnlands triploid mit 33 Chromosomen (SUOMALAINEN, 1940 a, S. 57; 1940 b, S. 82—84).

Zwei Weibchen aus Berlin-Buch wurden zytologisch untersucht. Auch sie waren triploid, es wurden nämlich in den Eiern in der Metaphaseplatte der Reifungsteilung 33 Chromosomen festgestellt.

Strophosomus melanogrammus. — Diese Art ist auf Åland im südwestlichen Finnland triploid. Die Chromosomenzahl variiert in den Oozyten von 31 bis 35. Als häufigste Chromosomenzahl ergibt sich 34 (SUOMALAINEN, 1940 a, S. 56; 1940 b, S. 78—80).

Auch von dieser Art wurden zwei in Berlin-Buch gesammelte Weibchen untersucht. Beide waren triploid. Von den fünf Eiern, die eine exakte Bestimmung der Chromosomenzahl gestatteten, wurden bei vier in der Metaphase der Reifungsteilung 34 Chromosomen konstatiert; das fünfte hatte 33 Chromosomen. Wie bei den finnischen Exemplaren, gewahrt man auch hier ein im Vergleich zu den übrigen deutlich kleineres Chromosom.

B. DIE TETRAPLOIDEN ARTEN UND RASSEN.

Von den zehn parthenogenetischen Rüsselkäferarten, deren Chromosomenverhältnisse in dieser Untersuchung näher behandelt werden, erwies sich nur eine als tetraploid.

Otiorrhynchus pupillatus. — Die Art ist in Lunz am See parthenogenetisch; die gesammelten 26 Exemplare waren alle Weibchen. Vier von diesen wurden zytologisch untersucht. Sie waren alle tetraploid. Wegen der grossen Zahl der Chromosomen war ihre exakte Zählung nur in zwei Metaphaseplatten der Reifungsteilung möglich. Beide enthielten 44 univalente Chromosomen (Fig. 21). Ausserdem wurden in mehreren Metaphaseplatten ungefähr 44 Chromosomen festgestellt. Die grösseren Chromosomen sind langgestreckt, in ihrer Mitte deutlich gebogen.

C. DIE PENTAPLOIDEN ARTEN UND RASSEN.

Eine von den untersuchten Arten erwies sich als pentaploid.

Barynotus moerens (= *elevatus*). — Zwei Weibchen aus Lunz am See wurden untersucht. Sie waren beide pentaploid mit 55 Chromosomen. Ungeachtet der recht grossen Chromosomenzahl gelang es mir in drei Metaphaseplatten der Reifungsteilung exakt die genannte Zahl von Chromosomen zu konstatieren (Fig. 22—23). Ausserdem waren in sechs Metaphaseplatten ungefähr 55 Chromosomen feststellbar. Dazu kommen noch einige Platten, die etwas über 50 Chromosomen zeigen. Die Pentaploidie wurde also bei dieser Art ganz einwandfrei festgestellt. Ganz wie bei der anderen, früher untersuchten *Barynotus*-Art, *B. obscurus* (vgl. SUOMALAINEN, 1940 a and b), sind die Chromosomen von *B. moerens* recht gross und zeigen oft ein deutliches, nahezu medianes Zentromer.

Wenngleich die Art in Finnland recht selten ist, gelang es mir nach eifrigem Suchen zwei *Barynotus moerens*-Weibchen (die Bestimmung dieser Exemplare ist von Herrn Dozent Dr. ERNST PALMÉN bestätigt worden) aus dem Botanischen Garten der Universität Helsinki zur Untersuchung zu bekommen. Leider war es nicht möglich, ihre Chromosomenzahl genau festzustellen. Sie scheinen jedoch viel weniger Chromosomen zu haben als die pentaploiden Exemplare aus Lunz. Die finnischen *Barynotus moerens*-Weibchen repräsentieren somit eine niedrigere Polyploidiestufe (vielleicht Triploidie?) als die untersuchten österreichischen Exemplare. Ich hoffe neues Material zu erhalten, um diese interessanten Chromosomenverhältnisse genauer untersuchen zu können.

D. DIE ORIENTIERUNG DER CHROMOSOMEN IN DER METAPHASEPLATTE BEI DEN PARTHENOGENETISCHEN ARTEN.

In den Eiern mancher von mir (SUOMALAINEN, 1940 a und b) früher untersuchten polyploiden Rüsselkäfer lässt sich ziemlich allgemein eine eigenartige Gonomerie wahrnehmen. Die Chromosomen können sich in der Metaphase der Reifungsteilung zu zwei oder sogar drei getrennte Platten anordnen. Drei Platten findet man jedoch nur bei tetraploiden Arten. Jede der Platten enthält im allgemeinen ein oder mehrere volle Genome. Diese getrennten Platten können weit voneinander entfernt liegen.

Auch bei den in der vorliegenden Untersuchung beschriebenen polyploiden Curculionidenarten wurde eine ähnliche Genomsonderung festgestellt. Bei allen sechs triploiden *Otiorrhynchus*-Arten wurden neben Eiern mit einer triploiden Metaphaseplatte auch solche Eier gefunden, die eine diploide und eine haploide Platte aufweisen. Die Zahl der letztgenannten Eier war jedoch im allgemeinen ziemlich gering, sie schwankte nämlich lediglich zwischen 2,5 % (*O. singularis*) und 12,5 % (*O. salicis*). (Dasjenige *O. salicis*-Weibchen, dessen Eier eine ganz unregelmässige Gruppierung der Metaphasechromosomen zeigen [vgl. S. 435], ist hierbei nicht berücksichtigt.) Nur bei *O. niger* sind ziemlich viele, nämlich 25 % der Eier mit Teilplatten zu finden. Beim tetraploiden *O. pupillatus* befanden sich unter 22 Eiern zwei mit einer triploiden und einer haploiden Platte und eines mit zwei diploiden Platten (das Weibchen mit »degenerierenden» Eiern [vgl. unten] scheidet hierbei aus) und beim pentaploiden *Barynotus moerens* befand sich unter 32 Eiern eines mit einer triploiden und einer diploiden Platte.

Eines von den untersuchten *Otiorrhynchus pupillatus*-Weibchen zeigt in seinem Ovarium mehrere Eier, deren Plasma Anzeichen einer beginnenden Degeneration aufweist. In diesen Eiern kann man 2—3 weit voneinander entfernt liegende degenerierende Chromosomengruppen wahrnehmen, die verhältnismässig wenig (eine haploide oder diploide Zahl?) Chromosomen enthalten. Es ist möglich, dass wir es hier mit solchen Fällen zu tun haben, in welchen die Teilplatten so weit voneinander entfernt liegen, dass eine normale Entwicklung des Eies nicht mehr möglich ist.

III. BESPRECHUNG DER ERGEBNISSE.

1. DIE VERSCHIEDENEN RASSEN EIN UND DERSELBEN ART MITEINANDER VERGLICHEN.

Oben (S. 426) ist bereits davon die Rede gewesen, dass man bei manchen Rüsselkäferarten sowohl eine bisexuelle als eine parthenogenetische Rasse kennt. Einige andere Arten vermehren sich nur parthenogenetisch; bei ihnen ist keine bisexuelle Rasse festgestellt worden. Doch sind in vielen solchen Fällen ganz naheverwandte bisexuelle Arten bekannt, die möglicherweise als die bisexuellen Gegenstücke der betr. Arten gelten könnten, obwohl sie als eigene Arten bezeichnet worden sind. Es hat mir bisher an Gelegenheit gefehlt, die Chromosomenverhältnisse solcher einander entsprechenden fortpflanzungsbiologisch verschiedenen Curculionidenrassen zu untersuchen. In vorliegender Arbeit sind jedoch sowohl die bisexuelle als die parthenogenetische Rasse dreier *Otiorrhynchus*-Arten, nämlich *O. niger*, *O. salicis* und *O. gemmatus*, zytologisch untersucht worden. Bei allen diesen Arten ist die bisexuelle Rasse diploid, und ihre Eier laufen zwei Reifungsteilungen durch. Die parthenogenetische Rasse ist dagegen polyploid (triploid); die Eier dieser Rasse machen nur eine Reifungsteilung, die Äquationsteilung, durch. In allen diesen Fällen ist die parthenogenetische Vermehrung also mit Polyploidie verbunden. Bemerkenswert ist auch, dass bei den Rüsselkäfern, wenn die Vermehrung parthenogenetisch wird, die Tetradenbildung wie auch die Chromosomenreduktion ausfallen.

Auch zytologisch verschiedene parthenogenetische Rassen wurden bei einigen Curculionidenarten konstatiert. So wurden bei *Otiorrhynchus scaber* zwei verschiedene parthenogenetische Rassen, nämlich eine triploide und eine tetraploide, festgestellt. Entsprechenden Verhältnissen begegnen wir bei *Barynotus moerens*, der neben einer penta-

ploiden parthenogenetischen Rasse eine andere parthenogenetische Rasse hat, die eine niedrigere Polyploidiestufe repräsentiert. Bei *Otiorrhynchus scaber* tritt in den Ostalpen in einigen ganz beschränkten Gebieten (Näheres auf S. 444) noch eine bisexuelle Rasse auf, die mit recht grosser Wahrscheinlichkeit diploid ist. *Otiorrhynchus scaber* ist somit eine Art, von der es wenigstens drei verschiedene Rassen gibt, nämlich eine diploide bisexuelle, eine triploide parthenogenetische und eine tetraploide parthenogenetische.

Bei manchen polyploiden Tieren tritt als Folgeerscheinung der Polyploidie Gigantismus auf; bei anderen wiederum ist solches nicht nachweisbar (Näheres z. B. bei FANKHAUSER, 1945, S. 48—50). HOFFMANN (1932) hat bezüglich zweier Arten der Curculionidengattung *Trachyphloeus*, *Tr. alternans* und *Tr. spinimanus*, festgestellt, dass bei ihnen die bisexuelle Rasse um die Hälfte kleiner als die parthenogenetische (und wahrscheinlich polyploide; vgl. VANDEL, 1932) ist. Die verschiedenen Rassen von *Otiorrhynchus niger*, *O. salicis* und *O. gemmatus* wurden leider erst bei der zytologischen Untersuchung festgestellt, als ein Vergleich ihrer Körpergrösse nicht mehr möglich war. Die beiden zytologisch verschiedenen parthenogenetischen Rassen von *Otiorrhynchus scaber* liessen sich dagegen in betreff ihrer Körpergrösse miteinander vergleichen. Wie aus Fig. 24 hervorgeht, ist die triploide Rasse aus Lunz am See deutlich etwas kleiner als die tetraploide aus Finnland; der Unterschied ist allerdings nicht gross.

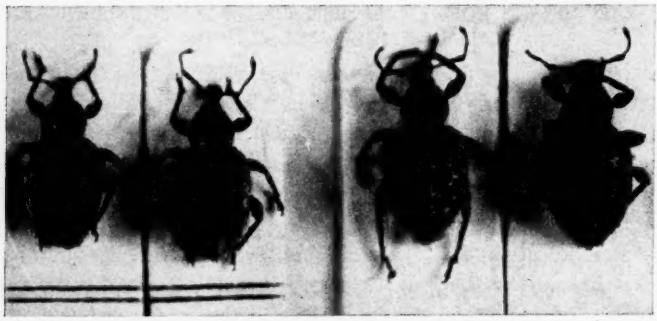

Fig. 24. Triploide *Otiorrhynchus scaber*-Individuen aus den Österreichischen Kalkalpen (Lunz am See) (links) und tetraploide finnische Individuen derselben Art (rechts). 4,5 ×. — Der Farbenunterschied der Käfer beruht auf der die ersteren bedeckenden feinen staubartigen Schicht.

Die Eier der verschiedenen Rassen zeigen in bezug auf ihre Grösse

— soweit man dies aus den Schnittpräparaten beurteilen kann — keine grösseren Unterschiede. Der Querdurchmesser der Eier der diploiden bisexuellen Rasse von *Otiorrhynchus niger* ist 0,55 mm und derjenige der Eier der triploiden parthenogenetischen Rasse 0,57 mm. Bei *O. salicis* sind die entsprechenden Werte 0,43 mm und 0,43 mm und bei *O. gemmatus* 0,61 mm und 0,65 mm (alles Mittelwerte von 10 reifen Eiern). Entsprechend liegt die Sache bei *O. scaber*; der Querdurchmesser der Eier der triploiden parthenogenetischen Rasse ist nämlich 0,37 mm und derjenige der tetraploiden Rasse ebenfalls 0,37 mm (Mittelwerte von 20 reifen Eiern). Die Grössenverhältnisse der Eier verschiedener Curculionidenrassen ähneln somit denjenigen bei *Solenobia*, deren verschiedene Rassen auch gleich grosse Eier haben (SEILER, 1936, S. 150).

2. DIE VERBREITUNG DER VERSCHIEDENEN RASSEN EIN UND DERSELBEN ART.

Wenn von zwei naheverwandten Arten oder von zwei Rassen ein und derselben Art die eine bisexuell und die andere thelytok parthenogenetisch ist, besitzen die beiden Arten bzw. Rassen gewöhnlich wenigstens zum Teil ihre eigenen Verbreitungsgebiete, und zwar oft so, dass die parthenogenetische Form die nördlichere ist (siehe z. B. VANDEL, 1928, S. 244 ff.; 1931, S. 174—200). Wegen der verschiedenen Verbreitung der parthenogenetischen Form werden derartige Fälle als geographische Parthenogenese bezeichnet (VANDEL, op. c.).

Die direkte Ursache zu der geographischen Parthenogenese ist wenigstens in den meist typischen Fällen jedoch nicht die Parthenogenese selbst, sondern die den parthenogenetischen Formen eigene Polyploidie. Wo die bisexuelle und die parthenogenetische Rasse eine deutlich verschiedene Verbreitung aufweisen, hat sich die letzterwähnte meistens als polyploid erwiesen. Besonders deutliche Beispiele von solchen Fällen bieten uns u. a. die parthenogenetischen Rassen von *Artemia salina* (ARTOM, 1911, 1931; GROSS, 1932; BARIGOZZI, 1934, 1935), *Trichoniscus elisabethae* (VANDEL, 1926, 1928, 1931, 1934), *Saga pedo* (MATTHEY, 1941, 1946; GOLDSCHMIDT, 1946), manchen Phasmiden (über diesbezügliche Literatur näher bei WHITE, 1945, S. 295), und *Solenobia triquetrella* (SEILER, 1923, 1927, 1939, 1942, 1943). Obwohl bei allen diesen Tieren die Polyploidie die eigentliche Ursache der geographischen Parthenogenese ist, ist anderseits zu bemerken, dass ihre Polyploidie gerade durch die Parthenogenese ermöglicht ist (Näheres bei SUOMALAINEN, 1940 b, S. 122 ff.).

Die verschiedene Verbreitung der diploiden bisexuellen und der ihr entsprechenden polyploiden parthenogenetischen Rasse ist bei manchen Curculionidenarten recht deutlich feststellbar. Ein ausgezeichnetes Beispiel dafür bietet *Otiorrhynchus dubius*. Diese Art hat eine typische sog. boreoalpine Verbreitung (SZÉKESSY, 1937, S. 582; HOLDHAUS und LINDROTH, 1939, S. 215—218). In ihrem borealen Verbreitungsgebiet (Grönland, Island, Färöer, Grossbritannien, Irland, Dänemark, Fennoskandien und Nordrussland) ist sie ausschliesslich parthenogenetisch und wenigstens in einem Teil des Gebietes polyploid (in Finnland hat sich nämlich die Art als tetraploid erwiesen [SUOMALAINEN, 1940 a, S. 57; 1940 b, S. 85—88]). In seinem alpinen Verbreitungsgebiet dagegen (in manchen mitteleuropäischen Gebirgen) ist *O. dubius*, soweit vorläufig bekannt, ausschliesslich bisexuell und somit recht wahrscheinlich diploid. (Siehe hierzu die Karte bei SUOMALAINEN, 1945, S. 189.)

Auch eine andere *Otiorrhynchus*-Art, *O. salicis*, eignet sich gut als Beispiel. Nach JAHN (1941, S. 368) pflanzt sich diese Art im grössten Teil ihres Verbreitungsgebietes, so in Nordeuropa, der Schweiz, in den Sudeten, Karpathen und auf der Balkanhalbinsel, parthenogenetisch fort; für die Alpen ist jedoch das Vorkommen der bisexuellen Rasse angegeben. JAHN erwähnt indessen zwei Fundorte (Königsstuhlgebiet, Goldeckgebiet) in den Ostalpen, wo die parthenogenetische Rasse festgestellt worden ist. Wie früher (S. 435) erwähnt wurde, kommen die beiden Rassen in Lunz am See nebeneinander vor; die bisexuelle Rasse erwies sich als diploid und die parthenogenetische als triploid.

Es ist klar, dass die bisexuelle Rasse bei allen diesen Arten als ursprünglich zu gelten hat. Diese Curculionidenarten bieten ein gutes Beispiel dafür, wie eine später entstandene polyploide parthenogenetische Form sich in ganz neue Gebiete auszubreiten vermocht hat. Sehr aufschlussreich in dieser Beziehung sind alle diejenigen *Otiorrhynchus*-Arten, von denen in den Alpen sowohl eine bisexuelle als auch eine parthenogenetische Rasse anzutreffen sind. JAHN (1941) erwähnt 8 solche Arten, nämlich:

O. foraminosus	*O. azaleae*	*O. auricomus*
O. chalceus	*O. alpicola*	*O. chrysocomus*
O. scaber	*O. salicis*	

Zu diesen sind noch wenigstens zwei Arten hinzuzufügen, nämlich *O. niger* und *O. gemmatus*, die beide in Lunz am See sowohl eine bisexuelle als eine parthenogenetische Rasse aufweisen. Die Verbreitung der beiden verschiedenen Rassen der zwei letztgenannten Arten ist jedoch noch

nicht näher bekannt. Dagegen ist die Verbreitung aller 8 von JAHN erwähnten Arten besonders in den Ostalpen dank Professor Dr. K. HOLDHAUS sehr gut bekannt.

Aus der Karte von JAHN (op. c.; vgl. auch SUOMALAINEN, 1945, S. 190) geht deutlich hervor, dass die bisexuellen Rassen der betreffenden *Otiorrhynchus*-Arten diejenigen Alpengebiete bewohnen, die während der letzten Vereisung (der Würm-Eiszeit) eisfrei gewesen sind (»Massifs de refuge»). Eine Ausnahme macht nur *O. salicis*, dessen bisexuelle Rasse heute auch in dem damals eisbedeckten Gebiet auftritt. Die parthenogenetischen Rassen aller Arten kommen wiederum fast ausnahmslos vor in Gebieten, die während der Würm-Eiszeit von Gletschern bedeckt waren. Nach HOLDHAUS (1929, S. 979) gab es in den östlichen und südlichen Teilen der Alpen während der letzten Vereisung ausgedehnte eisfreie Gebiete, in deren tiefen Lagen auch der Wald zu wachsen vermochte. Ganz wie heute gab es an den Ost- und Südrändern der Alpen auch damals infolge der günstigen Exposition besonders in geschützten Tälern warme und trockne (xerothermische) Lokalitäten, auf welchen sich die sogar relativ wärmefordernden Vertreter der Alpenfauna die Eiszeit hindurch erhalten konnten. Die Fauna dieser Gebiete ist auch heute noch weit artenreicher und vielgestaltiger als die der ehemals eisbedeckten, zentralen und nördlichen Teile der Alpen. Nach dem Rückzug der Gletscher sind die bisexuellen Rassen der in Frage stehenden *Otiorrhynchus*-Arten in diesen Gegenden zurückgeblieben. Nur die parthenogenetischen Rassen haben sich in die während der Eiszeit vergletscherten, klimatisch ungünstigeren Gebiete auszubreiten vermocht. Ganz entsprechende Verhältnisse hat SEILER (1943) bei der Psychide *Solenobia triquetrella* in der Schweiz festgestellt.

JAHN (1941, S. 370) erklärt die verschiedene Verbreitung der bisexuellen und der parthenogenetischen Rassen so, dass »die Männchen ungünstigen Umweltverhältnissen leichter unterliegen als die Weibchen, die eine grössere ökologische Valenz besitzen dürften. Damit darf das Auftreten weiter verbreiteter Arten in parthenogenetischen Formen in den hohen Lagen der Hochgebirge und des hohen Nordens erklärt werden». Nach ihr (l. c.) ist es auch ohne weiteres verständlich, dass »eine Rasse, die sich parthenogenetisch fortpflanzen kann, eine viel grössere Ausbreitungsfähigkeit besitzt als jene Formen, bei welchen zur Fortpflanzung Männchen benötigt werden».

Die verschiedene Verbreitung der beiden Rassen beruht m. E. jedoch nicht darauf, dass es die Parthenogenese an sich unmittelbar ermög-

lichte, ungünstigere klimatische Verhältnisse besser zu ertragen. Die Ursache ist eben in der den parthenogenetischen Formen eigenen Polyploidie zu suchen, die allerdings, wie auf S. 441 schon erwähnt wurde, gerade durch die Parthenogenese ermöglicht wird. Es ist nämlich sehr wahrscheinlich, dass die betreffenden parthenogenetischen Rassen auch der genannten Alpen-*Otiorrhynchus*-Arten polyploid sind. Bei *O. scaber* und *O. salicis* habe ich das ja bereits festgestellt, wie oben angeführt. Die Polyploidie verändert die Reaktionsnorm und das Lebensoptimum der parthenogenetischen Rasse, und zwar oft gerade so, dass dieselbe in neue, klimatisch ungünstigere Gegenden übersiedelt. Wie wir uns aber erinnern, wurden in Lunz am See bei drei *Otiorrhynchus*-Arten sowohl eine diploide bisexuelle als eine triploide parthenogenetische Rasse gefunden. Dies kann (wenigstens in bezug auf *O. salicis*) davon herrühren, dass die verschiedenen Rassen natürlich in ihrem gemeinsamen Grenzgebiet nebeneinander vorkommen können. Auch wenn die polyploide parthenogenetische Rasse jung ist und noch nicht genug Zeit gehabt hat, in neue Gegenden überzusiedeln, können die beiden Rassen an ein und demselben Ort gemeinsam leben. Ob die polyploiden Rassen der betreffenden Curculionidenarten auch in Lunz am See den diploiden bisexuellen gegenüber in bezug auf ihre Standortsansprüche verschieden sind, z. B. in höheren Lagen leben, kann ich nicht entscheiden, weil diese verschiedenen Rassen leider erst bei der zytologischen Untersuchung festgestellt wurden.

Bemerkenswert ist, dass die Verbreitungsverhältnisse der polyploiden parthenogenetischen Tiere sehr an diejenigen mancher polyploiden Pflanzen erinnern; über diese Gegenseitigkeit sei des näheren auf die Anführungen von SUOMALAINEN (1940 b, S. 115—116) und VANDEL (1940, S. 95—97) verwiesen. Hier möge nur hervorgehoben werden, dass die Verbreitung der verschiedenen Formen von *Biscutella laevigata* in den Alpen (MANTON, 1934) in hohem Grade derjenigen der *Otiorrhynchus*-Rassen entspricht. Ganz ähnliche Verhältnisse liegen z. B. auch nach ANDERSON, WOODSON und SAX bei verschiedenen amerikanischen *Tradescantia*-Arten vor (DOBZHANSKY, 1941, S. 228).

Oben ist bereits davon die Rede gewesen, dass es von *Otiorrhynchus scaber* wenigstens drei verschiedene Rassen gibt, nämlich eine diploide bisexuelle, eine triploide parthenogenetische und eine tetraploide parthenogenetische. Es erhebt sich die Frage, wie weit diese Rassen verbreitet sind. Die bisexuelle Rasse tritt nur in einigen ganz beschränkten Gebieten in den Ostalpen (Umgebung von Graz, Wechselgebiet, Koralpen und Bürgeralm; vgl. JAHN, 1941, S. 367) auf. In den Österreichi-

schen Kalkalpen (Lunz am See) ist die Art triploid und in Finnland tetraploid.

Wie weit diese verschiedenen parthenogenetischen Rassen verbreitet sind, kann in gewissem Masse aus der Grösse der in verschiedenen Gegenden eingebrachten Sammlungsexemplare geschlossen werden, die triploiden Exemplare sind nämlich ein wenig kleiner als die tetraploiden (vgl. S. 440); irgendwelche sicheren Folgerungen können hieraus jedoch nicht gezogen werden. Einzelne Individuen beweisen in dieser Hinsicht nichts, es müssen mehrere aus demselben Ort vorliegen. Ich habe aus dem Riesengebirge und den Beskiden eine ganze Anzahl von *O. scaber*-Individuen gesehen; sie scheinen in ihrer Grösse mehr an die lunzischen als an die finnischen Individuen zu erinnern. Dies weist darauf hin, dass die triploide Rasse wenigstens in den mitteleuropäischen Gebirgsgegenden, vielleicht auch anderswo in Mitteleuropa die vorherrschende Form von *O. scaber* ist. Es ist möglich, dass die Art in Mitteleuropa triploid und in Nordeuropa tetraploid ist.

Eine endgültige Entscheidung dieser Frage sowie mancher anderen interessanten, auf die Parthenogenese und Polyploidie der Rüsselkäfer bezüglichen Probleme ist erst möglich, wenn wir viel mehr als heute von den Chromosomenverhältnissen dieser Tiere in verschiedenen Gegenden wissen.

3. DIE ENTSTEHUNG DER VERSCHIEDENEN RASSEN BEI DEN CURCULIONIDEN.

Obwohl die Parthenogenese und die Polyploidie bei den Tieren sehr oft in Verbindung miteinander auftreten, können wir jedoch nicht ohne weiteres voraussetzen, dass sie gleichzeitig entstehen. Das zwischen ihnen bestehende Abhängigkeitsverhältnis kann auch davon herrühren, dass die Parthenogenese bei Tieren die Polyploidie ermöglicht. Suchen wir nach den Ursachen der Polyploidie und Parthenogenese bei den Tieren, so müssen wir anderseits stets die oft geäusserte Vermutung in Betracht ziehen, dass diese bei den verschiedenen Formen in verschiedener Weise entstehen können. VANDEL (1926, 1928, 1931) z. B. nimmt an, dass die Parthenogenese und die Polyploidie (Triploidie) bei *Trichoniscus elisabethae* am wahrscheinlichsten gleichzeitig als Folge der Befruchtung eines abnormen diploiden Eies entstanden sind. Nach ihm bewirkt die auf diese Weise zustandegekommene Triploidie ein Unterbleiben der Konjugation und der Reduktion im Ei, und es entsteht ein Vorkern, der keine Befruchtungsneigung zeigt. Infolgedessen entwik-

kelt sich das Ei parthenogenetisch. Bei *Artemia salina* (ARTOM, 1911, 1931; GROSS, 1932, 1935) und *Solenobia triquetrella* (SEILER, 1923, 1927, 1942, 1943) ist die Parthenogenese jedoch sicher früher als die Polyploidie entstanden. Von beiden diesen Tieren kennt man eine diploide bisexuelle Rasse, aus welcher die polyploiden parthenogenetischen Rassen durch automiktische Vorgänge entstanden sind. Aus Obigem geht hervor, dass die polyploiden parthenogenetischen Rassen der obenerwähnten Tiere sowie die übrigen früher untersuchten entsprechenden Tierformen als autopolyploid festgestellt oder vorausgesetzt worden sind.

Mit den Rüsselkäfern sind bisher keine eigentlichen Versuche zur Beleuchtung der Entstehung der verschiedenen Rassen angestellt worden. Ihre Züchtung ist nämlich recht schwierig, weil die meisten Vertreter der Unterfamilien Otiorrhynchinae und Brachyderinae als Larven rhizophag unter der Erdoberfläche leben. Was ich im folgenden vorbringe, trägt darum lediglich den Charakter von Hypothesen.

Versuchen wir es nun, die Entstehung der verschiedenen parthenogenetischen Curculionidenrassen zu erklären, so kommen zunächst zwei prinzipiell verschiedene Alternativen in Frage.

Die diploiden bisexuellen Rassen sind bei den Rüsselkäfern, wie auch bei den anderen parthenogenetischen Tieren, sicher ursprünglich; die parthenogenetischen Rassen sind aus ihnen entstanden. Es ist möglich, dass bei den Curculioniden ähnlich wie z. B. bei *Solenobia triquetrella* (SEILER, op. c.) aus der bisexuellen Rasse zuerst eine diploide parthenogenetische hervorgeht, d. h. zunächst eine Veränderung der Vermehrungsart eintritt, ohne dass sich die Chromosomengarnitur veränderte. Dazu könnte jemand bemerken, man kenne bisher nur eine diploide parthenogenetische Curculionidenart (*Polydrosus mollis*), während polyploide parthenogenetische Formen von insgesamt 15 Arten bekannt sind. Die Seltenheit der diploiden parthenogenetischen Rassen kann möglicherweise davon herrühren, dass sie wie die diploiden bisexuellen Rassen mancher Arten (z. B. *Otiorrhynchus scaber*) innerhalb ganz beschränkter Gebiete auftreten und darum schwer zu finden sind. SEILER (op. c.) hat die diploiden Rassen von *Solenobia triquetrella*, sowohl die bisexuelle als die parthenogenetische, in der Schweiz nur an wenigen Orten gefunden — alle in Gebieten gelegen, die entweder als Nunataker aus dem einstigen Vereisungsgebiet der Alpen aufragten oder in dessen Aussenrandzonen gelegen waren — während die tetraploide parthenogenetische Rasse weit verbreitet ist. SEILER hält die diploide bisexuelle Rasse für ursprünglich. Aus ihr ist zuerst die diploide parthenogenetische und aus dieser wiederum die tetraploide parthenogeneti-

sche Rasse entstanden. Es ist SEILER (1942, S. 517—518) bei seinen Versuchen in der Tat gelungen, aus der bisexuellen Rasse eine parthenogenetische hervorgehen zu lassen. Er hat neuerdings (1943, S. 696) auch eine Rasse gefunden, bei der die Parthenogenese fakultativ ist. Die diploide parthenogenetische Rasse ist also bei *Solenobia* nur eine kurzfristige Zwischenstufe, über welche aus der bisexuellen Rasse eine tetraploide parthenogenetische entstanden ist.

Bei den Rüsselkäfern kann es sich möglicherweise ähnlich verhalten. Es ist möglich, dass bei den Curculioniden auch diploide parthenogenetische Rassen vorkommen oder vorgekommen sind, wenn auch in beschränkten Gebieten. FAGERLIND (1944, S. 192—193) bemerkt, dass dieser Umstand, »dass vielen apomiktischen Polyploiden nicht apomiktische oder nicht einmal sexuelle Diploide entsprechen, kann auf einer reinen Konkurrenzerscheinung beruhen. Ist eine Entsprechung vorhanden, so macht diese oft den Eindruck, von Reliktcharakter zu sein. Sie ist auf ein kleines Verbreitungsgebiet beschränkt und weist oft deutliche Zeichen auf, dass sie in Rückgang begriffen ist, während die apomiktische Polyploide einen vitaleren Eindruck macht und ein relativ grösseres Verbreitungsgebiet hat». Demnach liegt ja grosse Gefahr vor, dass »zunächst die diploiden Apomikten und dann auch die sexuellen Formen aussterben. Man hat daher wenig Möglichkeiten, sie anzutreffen». Meines Erachtens wären die eventuellen diploiden parthenogenetischen Curculionidenrassen dem *Solenobia*-Fall entsprechend in denselben Gegenden zu finden wie die bisexuellen Rassen, also in erster Linie in mittel- und südeuropäischen Gebirgsgegenden. Die zytologischen Verhältnisse bei *Polydrosus mollis* (SUOMALAINEN, 1940 a und b) zeigen jedenfalls, dass es unter den Rüsselkäfern wirklich auch diploide parthenogenetische Formen gibt.

Es ist jedoch zu bemerken, dass der Übergang von der bisexuellen Rasse zur parthenogenetischen bei den Curculioniden ganz andersartige Veränderungen im Chromosomenmechanismus als bei *Solenobia* voraussetzt. Die Chromosomenverhältnisse von *Solenobia triquetrella* (SEILER, op. c.) sind derart, dass der Übergang zur parthenogenetischen Vermehrung verhältnismässig leicht ist. Die Eier auch der parthenogenetischen Rassen dieser Art machen nämlich zwei Reifungsteilungen durch. Die normale Chromosomenzahl wird dadurch wiederhergestellt, dass nach den ersten Furchungsteilungen zwei Kerne miteinander verschmelzen. Die bei der Befruchtung vor sich gehende Verschmelzung der Kerne der verschiedenen Individuen, die Amphimixis, wird bei der Parthenogenese von *Solenobia* durch die Verschmelzung der Kerne des-

selben Individuums, durch Automixis, ersetzt. Die tetraploide Rasse ist wiederum aus der diploiden parthenogenetischen gewiss auf die Weise entstanden, dass bei den ersten Furchungsteilungen nicht nur ein, sondern zwei aufeinanderfolgende Kernverschmelzungen eingetreten sind. Bei den Curculioniden liegt die Sache anders. Weil bei ihnen die parthenogenetischen Eier nur eine Reifungsteilung, die Äquationsteilung, durchmachen, fallen bei ihnen, wenn die Vermehrung parthenogenetisch wird, die Tetradenbildung wie auch die Chromosomenreduktion gänzlich aus.

Nehmen wir an, dass auch bei den Curculioniden zuerst diploide parthenogenetische Rassen entstehen, so könnten sich aus jenen natürlich tetraploide durch Verdoppelung der diploiden Chromosomengarnitur entwickeln. Die Entstehung triploider und pentaploider Rassen dagegen ist auf diesem Wege schwer zu verstehen. Bei der Erörterung der Entstehung triploider Rassen könnte man ausser von einer diploiden auch von einer tetraploiden parthenogenetischen Rasse ausgehen. Es ist zu bemerken, dass (Näheres auf S. 438) die Metaphasechromosomen in den Eiern mancher polyploiden Curculioniden oft nicht in einer, sondern in mehreren Platten auftreten, wenn auch derart, dass in jeder Platte im allgemeinen ein oder mehrere volle Genome vorkommen. Da diese Teilplatten ziemlich weit voneinander entfernt liegen können, wäre es denkbar, dass irgendein Genom von der aus den übrigen Chromosomen bestehenden Gruppe und somit auch von dem Kern des mit seiner Entwicklung einsetzenden Eies endgültig ausgeschlossen würde. Könnte man doch dadurch von einer tetraploiden Rasse leicht zu einer triploiden gelangen.

Gegen eine solche Möglichkeit spricht jedoch ein schwerwiegender Umstand. Wenn das wirklich geschähe, so müsste es z. B. von *Otiorrhynchus dubius* in Finnland sowohl triploide als tetraploide Individuen geben, da etwa 45 % von den Eiern der finnischen Individuen Teilplatten enthalten. Es sind aber alle bisher von mir untersuchten finnischen *O. dubius*-Individuen durchgehends tetraploid gewesen. Die Entstehung triploider Rassen aus tetraploiden durch Teilplattenbildung ist also nicht wahrscheinlich. Der Umstand, dass *O. pupillatus*-Eier mit weit voneinander liegenden Teilplatten (S. 439) zu degenerieren beginnen, deutet darauf hin, dass solche Eier nicht entwicklungsfähig sind.

Es wäre auch denkbar, dass eine triploide Rasse durch Kreuzung zweier anderer Rassen entstehen könnte. SEILER (op. c.) ist es gelungen, die diploide bisexuelle und die tetraploide parthenogenetische Rasse von *Solenobia triquetrella* miteinander zu kreuzen, wobei triploide Indivi-

duen entstanden. Bei *Solenobia* kann jedoch wegen der weiblichen Heterogametie der Schmetterlinge keine beständige triploide Rasse entstehen, denn die triploiden Individuen sind infolge des veränderten quantitativen Verhältnisses zwischen Geschlechtschromosomen und Autosomen intersexuell.

SEILER (1945) hat neuerdings auch ein triploides intersexuelles Freilandtier von *Solenobia alpicolella* gefunden, das nach ihm am wahrscheinlichsten als Nachkomme eines überreifen begatteten Weibchens entstanden ist. In den Eiern der überreifen bisexuellen Weibchen wird nämlich die Chromosomenzahl möglicherweise durch Automixis zur Diploidie aufreguliert; wird weiter ein solches Ei bei der Ablage besamt, so resultiert die Triploidie und damit Intersexualität.

Weil bei den Curculioniden das Weibchen homogametisch ist, sind die triploiden Weibchen nicht intersexuell. Somit können bei ihnen beständige triploide parthenogenetische Rassen vorkommen. Doch kann bei den Rüsselkäfern eine triploide Rasse als ähnliche Kreuzung wie bei den Solenobien nicht entstehen. Weil die Eier aller *Solenobia*-Rassen zwei Reifungsteilungen durchlaufen, so sind die Eier eines tetraploiden *Solenobia*-Weibchens diploid; wird ein solches Ei besamt, so entsteht eine triploide Zygote. Die Eier der parthenogenetischen Curculioniden machen dagegen nur die Äquationsteilung durch; in ihren Eiern findet also keine Chromosomenreduktion statt. Bei etwaiger Kreuzung einer tetraploiden parthenogenetischen Rasse mit einer diploiden bisexuellen müsste also eine pentaploide Rasse entstehen. Eine solche wurde ja bei *Barynotus moerens* in den Österreichischen Kalkalpen gefunden (S. 437). Eine triploide Rasse könnte dagegen aus der Kreuzung einer diploiden parthenogenetischen Rasse mit einer diploiden bisexuellen entstehen, denn dabei vereinigten sich eben ein diploides Ei und eine haploide Samenzelle.

Das Vorhandensein pentaploider Rassen bei den Curculioniden deutet darauf hin, dass die parthenogenetischen Rassen sich in der Natur wirklich mit den bisexuellen kreuzen können. Die Entstehung einer pentaploiden parthenogenetischen Curculionidenrasse ist nämlich am wahrscheinlichsten auf die Befruchtung eines tetraploiden Eies zurückzuführen (vgl. z. B. MÜNTZING, 1944, S. 633; FANKHAUSER, 1945, S. 36), und dies wäre am leichtesten eben bei der Kreuzung einer tetraploiden parthenogenetischen Rasse mit einer diploiden bisexuellen denkbar. Und könnte sich einmal die diploide bisexuelle Rasse mit polyploiden parthenogenetischen Rassen kreuzen, so wäre eine Kreuzung der

beiden diploiden Rassen, der bisexuellen mit der parthenogenetischen, natürlich eher möglich.

Die relative Häufigkeit der triploiden parthenogenetischen Rassen — von den von mir untersuchten parthenogenetischen Curculionidenrassen oder -arten ist ja eine diploid, 11 sind triploid, 4 tetraploid und eine pentaploid — zeugt dafür, dass die Triploidie leichter als die höheren Polyploidiestufen entsteht. Dies könnte möglicherweise eben zum Teil darauf beruhen, dass die beiden diploiden Rassen, weil sie in denselben Gegenden vorkommen, bessere Voraussetzungen zur Kreuzung miteinander haben als eine diploide bisexuelle und eine polyploide parthenogenetische, die oft verschiedene Gegenden bewohnen.

Der Umstand, dass die höheren Polyploidiestufen bei den Curculioniden verhältnismässig selten sind, kann wenigstens zum Teil auch davon herrühren, dass entwicklungsphysiologische Bedingungen die obere Grenze der Polyploidiestufe ziemlich niedrig halten.

Bemerkenswert ist, dass der Kreuzung verschiedener Rassen auch keine morphologischen Hindernisse im Wege stehen, denn SZÉKESSY (1937, S. 579—581) hat festgestellt, dass der Bauplan des weiblichen Kopulationsapparates wenigstens einiger polyploiden parthenogenetischen Curculionidenformen (z. B. *Otiorrhynchus ovatus*) »keine sekundären Modifikationen aufweist, sondern dass er trotz der abgeänderten Fortpflanzungsweise seine ursprüngliche Ausgestaltung beibehalten hat». Ferner ist zu bemerken, dass die jungen weiblichen Imagines bei sowohl bisexuellen als parthenogenetischen Curculioniden in ihren Ovarien noch nicht reife Eier haben. Somit hätten auch die parthenogenetischen Weibchen vor der Eiablage genug Zeit, mit Männchen der bisexuellen Rasse zu kopulieren. Was die sich eventuell kreuzenden Rassen betrifft, könnten m. E. als solche ausser den verschiedenen Rassen derselben Art vielleicht auch solche nahestehender Arten in Frage kommen. Im letzteren Falle hätten wir es nicht mehr mit Autopolyploidie, sondern mit Allopolyploidie zu tun.

Eine zweite, von der im vorhergehenden erörterten prinzipiell ganz verschiedene Möglichkeit, die Entstehung der polyploiden parthenogenetischen Curculionidenrassen zu erklären, wäre die Annahme, dass die Parthenogenese und die Polyploidie bei den Curculioniden gleichzeitig entstehen. Bei den Amphibien ist festgestellt worden (Näheres bei FANKHAUSER, 1942 und 1945), dass extreme Temperaturbedingungen — sowohl nach oben als nach unten hin — in den abgelegten, sich im Metaphasestadium der 2. Teilung befindlichen Eiern die 2. Reifungsteilung verhindern, wodurch diploide Eier entstehen. Wenn solche Eier

befruchtet sind, entwickeln sie sich zu triploiden Tieren. So kann z. B. rasche Temperaturerniedrigung während der Laichzeit dazu führen, dass auch in der Natur triploide Individuen entstehen.

FANKHAUSER (op. c.) hat auch einige tetraploide und einige pentaploide Urodelenlarven gefunden. Die Entstehung der ersteren könnte nach ihm (1945, S. 42) so erklärt werden, dass die extremen Temperaturbedingungen die Trennung der Teilungsprodukte der Chromosomen in der 1. Furchungsteilung des normalen befruchteten Eies verhindern, wodurch die diploide Chromosomengarnitur verdoppelt wird. Ein pentaploides Tier wiederum könnte durch Befruchtung eines tetraploiden Eies entstehen. »A tetraploid gamete may originate in various ways, e. g., by suppression of both maturation division and inclusion of all tetrads in a single nucleus» (1945, S. 36). Bei den Amphibien führt die Polyploidie jedoch nicht zu Parthenogenese.

Es ist nicht ausgeschlossen, dass auch bei den Curculioniden etwas Ähnliches geschieht. Die Entstehung polyploider parthenogenetischer Curculionidenrassen als Folge solcher Ereignisse setzt jedoch notgedrungen voraus, dass gleichzeitig mit der Polyploidie eine solche Genkombination entsteht, die sowohl das Ausbleiben der Tetradenbildung und der Chromosomenreduktion, also das Ausbleiben der einen von den zwei Reifungsteilungen, als die parthenogenetische Entwicklung des Eies verursacht. Es ist zu bemerken, dass die unbefruchteten Eier des Seidenspinners, *Bombyx mori*, nach einer kurzen Wärmebehandlung sowohl nur eine einzige Reifungsteilung durchlaufen, die eine Äquationsteilung ist, als sich parthenogenetisch entwickeln (ASTAUROV, 1940).

Es ist mir eine angenehme Pflicht, der Kaiser Wilhelm-Gesellschaft zur Förderung der Wissenschaften für die wertvolle Einladung, als Gast der Gesellschaft in ihren Instituten zu arbeiten, meinen aufrichtigen Dank auszusprechen. Diese Einladung ermöglichte es mir auch, während eines Monats in der Biologischen Station Lunz der Kaiser Wilhelm-Gesellschaft zu arbeiten, woselbst der grösste Teil des hier behandelten Materials eingesammelt ist. Besonders danke ich dem Direktor der Abteilung H. Bauer am Kaiser Wilhelm-Institut für Biologie, Herrn Doktor HANS BAUER, der mir sowohl während unseres gemeinsamen Aufenthalts in Lunz am See als während meines ganzen Deutschlandsverweils stets grösste Freundlichkeit erwies, desgleichen

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SUMMARY.

The present investigation deals with the chromosome relations of thirteen bisexual and ten parthenogenetic weevils, Curculionidae.

All the investigated bisexual species — they all belong to the genus *Otiorrhynchus* — have the same chromosome number ($2n = 22$). The males are heterogametic, belonging to the *XY* type; the females are homogametic. In all species bivalent formation takes place normally. In them, chiasmata are clearly visible and become symmetrical and terminalized simultaneously with the contraction of the chromosome. Consequently, typical rod-bivalents with one terminalized chiasma, and ring-bivalents with two chiasmata, are formed.

All the parthenogenetic weevils dealt with here are polyploid. Among them there are triploid, tetraploid and pentaploid species. *Otiorrhynchus niger* (from the Austrian Alps), *O. scaber* (from the Austrian Alps), *O. singularis* (from Helsinki and Berlin), *O. salicis* (from the Austrian Alps), *O. sulcatus* (from Helsinki), *O. gemmatus* (from the Austrian Alps), *Sciaphilus asperatus* (from Berlin) and *Strophosomus melanogrammus* (from Berlin) are triploid. *Otiorrhynchus pupillatus* (from the Austrian Alps) is tetraploid and *Barynotus moerens* (from the Austrian Alps) pentaploid. The egg-cells pass through one maturation division only, which is equational. In most parthenogenetic species the chromosomes at the metaphase of the maturation division may be arranged in two or even three different plates, each plate containing one or more complete sets of chromosomes.

Including the weevils studied by the author previously (SUOMALAINEN, 1940 a and b) the chromosome number of 17 parthenogenetic species and races is known. Of these 1 is diploid, 11 triploid, 4 tetraploid and 1 pentaploid.

Three species, viz. *Otiorrhynchus niger*, *O. salicis* and *O. gemmatus*, occur in the Austrian Alps (in Lunz am See) as well as a diploid bisexual as a triploid parthenogenetic race.

Of *Otiorrhynchus scaber*, a triploid parthenogenetic race is met with in the Austrian Alps and a tetraploid parthenogenetic race in Finland. It is possible that the former is distributed over Central Europe (at least in mountains) and the latter over North Europe. Of this species,

too, a bisexual, probably diploid race is known from some localities in the East Alps.

Of such *Otiorrhynchus* species as occur in the Alps both as a bisexual and a parthenogenetic race, the bisexual race is usually met with in habitats which were free from ice during the Würm Glaciation (»Massifs de refuge»). The parthenogenetic race, again, is distributed in areas which were then covered with ice. This depends at least mainly on the polyploidy of the parthenogenetic races. The same cause has also been active elsewhere in giving the parthenogenetic races a distribution different from that of the bisexual races.

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INHALT.

Seite

I. Material und Methoden 426

II. Die Chromosomenverhältnisse bei den untersuchten Curculioniden 428

1. Die bisexuellen Arten und Rassen 428
A. Die Spermatogenese 428
Spermatogonienchromosomen 428
Tetradengenese 430
Reifungsteilungen 431
B. Die Oogenese 431
Reifungsteilungen 431

2. Die parthenogenetischen Arten und Rassen 432
A. Die triploiden Arten und Rassen 433
Otiorrhynchus niger 433
Otiorrhynchus scaber 433
Otiorrhynchus singularis 435
Otiorrhynchus salicis 435

Seite

Otiorrhynchus sulcatus 436
Otiorrhynchus gemmatus 436
Sciaphilus asperatus 436
Strophosomus melanogrammus 437
B. Die tetraploiden Arten und Rassen 437
Otiorrhynchus pupillatus 437
C. Die pentaploiden Arten und Rassen 437
Barynotus moerens (= *elevatus*) 437
D. Die Orientierung der Chromosomen in der Metaphaseplatte bei den parthenogenetischen Arten 438

III. Besprechung der Ergebnisse 439
1. Die verschiedenen Rassen ein und derselben Art miteinander verglichen 439
2. Die Verbreitung der verschiedenen Rassen ein und derselben Art 441
3. Die Entstehung der verschiedenen Rassen bei den Curculioniden 445

Summary 452
Zitierte Literatur 453

STUDIES ON THE CAMPHOR REACTION OF YEAST

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I. INTRODUCTION.

IT has long been known that various *Saccharomyces* species under certain conditions may change their normal mode of growth and propagation. Instead of a bud growing out, which is abstricted after having reached a certain size, an oblongate tube is formed which is not separated from the mother cell. Brewers and technical workers have often observed such so-called involution forms in aging yeast cultures in connection with the autolysis phenomenon.

One factor which has been found to be of importance for the cellular shape of yeast is the temperature. Usually it has turned out that colder conditions favour a longer and narrower cell-form, while warmth causes the cells to become rounder and isodiametric. HANSEN (1886) shows that low temperature may give rise to associations of mycelium-like cells in such *Saccharomyces* species as, under high temperatures, give rounded, solitary cells. ZIKES (1920) studies the influence of temperature on different yeast genera and observes changes in cell-shape, »Modifikationen, d. h. flüchtige Varietäten», if the yeast is cultivated under abnormal temperatures. These aberrants disappear again after a short period of optimal temperature. He points out that the typical reaction is that at low temperature the yeast partly acquires a longer cell-shape, partly shows a tendency to hang together in colonies.

Certain yeast species are also characterized normally by long and narrow cells which preferably grow in colonies. Also within species which normally have a rounded cell-shape and live as solitary cells there may be found genotypes with greater or less tendency to grow like the former type. Thus, in *Saccharomyces anomalus*, by selecting one hypha-bearing cell BARBER (1907) succeeded in breeding a strain which continued to maintain this quality. The cells formed hyphae at the end of which normal cells could again bud off.

On comparing haploid and diploid yeast of the same genotype LINDEGREN (1944) found that the haploids showed greater tendencies

to an extended hypha-like growth: »Extremely thin elongated cells are also found in haploid cultures. Much of this variation is simply due to environmental conditions. Both bloated cells and long, thin cells are generally characteristic of haploid cultures. Many haploid cultures show a predominance of one or the other type, but these types are generally absent from diploid cultures» (l. c., p. 157).

SEGAL (1938, 1939) observed a regular appearance of morphological aberrations in the yeast cells after treatment with fusel oil and certain higher alcohols. He describes abnormal budding giving rise to cell-chains, where mother cells and daughter cells do not separate, and also deals with the origin of oblongate cells, beacher-like cells, etc.

The first worker to be able experimentally to produce a high and regular frequency of these aberrant involution forms is BAUCH (1941). He describes the peculiar behaviour of the yeast cells under influence of camphor as follows: »Lässt man Campherdämpfe auf die Hefe einwirken . . ., so zeigt sich bei geeigneter Dosierung ein überraschender morphogenetischer Einfluss dieser Substanz. Nach anfänglicher Wachstumshemmung beginnt ein Teil der überlebenden Zellen keimschlauchähnliche Auswüchse zu bilden, die schliesslich zu kleinen, meist bizarren Mycelien auswachsen . . . Die Reaktion wird sowohl vom rechtsdrehenden natürlichen Campher, wie vom racemischen synthetischen Campher ausgelöst. Dagegen sind alle bisher untersuchten Campherderivate und dem Campher nahestehenden Verbindungen wirkungslos geblieben» (1943 a, p. 46).

By taking progeny from camphor-treated cells BAUCH has also succeeded in producing giant and super-giant types of yeast, which he considers to be polyploids. Although he has not obtained the typical camphor reaction with any other substances, he reports the induction of polyploidy after manifold different treatments with c-mitotic, carcinogenic and growth-promoting substances. In a later paper (1943 b) BAUCH shows the origin of constant giant races after radium irradiation as well.

With the use of BAUCH's technique SUBRAMANIAM (1945) induced mycelium growth in yeast and also obtained tetraploid and octoploid cells. THAYSEN and MORRIS (1943) have induced giant growth and probable tetraploidy in *Torulopsis utilis* by using camphor. They also found a similar response after treatment with some other camphor derivatives.

Since 1942 a co-operation has been going on between the Central Laboratory of the Stockholm Brewery Co. and the Cyto-genetic Labor-

atory of the Swedish Seed Association. Special attention has been directed to the camphor reaction and the conditions for the origin of natural involution forms. Thus, LEVAN and SANDWALL (1943) determined the effective concentrations of camphor and borneol in giving camphor reaction. Later on I tested a fairly large number of chemicals as to their »camphor activity». Although great differences in efficiency were found between different substances, I found some activity in almost all of them, independently of their chemical constitution. Various circumstances, however, such as lethality and toxicity often make the demonstration of the reaction difficult. A short report (LEVAN, 1944) gives some data concerning the camphor activity of aliphatic alcohols. This paper is an attempt to put the camphor reaction into its wider connection with other similar vital reactions.

Studies on this problem have gone on for different periods. The experimental work has been done at the Central Laboratory and the cytological analysis at Svalöf. My conclusions of 1944 have gained further support. The present paper will give the material underlying the conclusions, as far as it has appeared up to now. The empirical rule which various workers have found to be valid in toxic and narcotic reactions, viz. a correlation between certain physical properties of the substances and their activity, has been studied in my experiments on yeast.

The present work is based on experimental series with a certain yeast clone, viz. the commercial variety of beer yeast (bottom yeast) of the Stockholm Breweries. As culture medium use has been made of the standardized type of wort generally used at the Central Laboratory. The possibly somewhat unfavourable condition of using wort instead of a synthetic culture medium in this kind of work was thought to be counterbalanced by the great advantage that the normal behaviour of the yeast in question in this wort type was studied and well-known long ago and that this wort is known to furnish optimal conditions for the yeast. In each substance tested the yeast was treated with a series of concentrations from saturated solution down to such dilutions as do not give any visible effect. In some series the rather comprehensive manner of proceeding described by LEVAN and SANDWALL (l. c.) was employed, each treatment being made in 100 cc of wort, the cell numbers being counted on different occasions in BÜRKER's counting chamber. Many experiments were more orientating and were made in wort quantities of 10 cc in small FREUDENREICH flasks. The poison effect was often estimated qualitatively without any

exact counts. It was realized that such estimations could be made fairly correctly if slides with the treated yeast were compared with slides from the control cultures. Exact cell numbers given below are always based on countings in BÜRKER's chamber, $^1/_{10}$ mm^3 having been counted on each occasion. When determining the frequency of camphor colonies each colony was recorded as one »individual» irrespective of its cell number.

Of certain substances that did not give lethal effect in saturated solution even solutions with various excesses of undissolved substance were tested. It was already known from our treatments with camphor and borneol (LEVAN and SANDWALL, l. c.) that an increased amount of precipitate may give an increased effect of both camphor reaction and toxicity. Similar conditions are also found in certain c-mitotic treatments (LEVAN and ÖSTERGREN, 1943, pp. 389—390).

The present investigations have been financed by the Stockholm Brewery Co., and the co-operation between the Central Laboratory and the Cyto-genetic Laboratory has been constantly supported by Dr. HARRY LUNDIN, Scientific Leader of the Stockholm Brewery Co., and Professor ÅKE ÅKERMAN, Director of the Seed Association. The staff of the Central Laboratory has in every way helped my work, and I am especially indebted to Mr. C. G. SANDWALL, microbiologist, and Mr. E. SANDEGREN, chief chemist of the laboratory. Drs. E. STEINEGGER and G. ÖSTERGREN have read the manuscript.

II. THE MORPHOLOGY OF THE CAMPHOR REACTION.

1. CELLULAR MORPHOLOGY.

The opinion of BAUCH that the camphor reaction is a property peculiar to camphor has not been found to hold true. The reaction is induced by most organic substances tested, aliphatic, alicyclic, monocyclic aromatic. In my experiments bicyclic and polycyclic aromatic compounds, among which, it is true, only few have been tested, have been without visible effect. The general type of the reaction is subject to considerable variation between different substances and groups of substances. It only rarely grows as complete and extreme as in camphor.

In accordance with the practice at the colchicine reactions, which are induced by a great many substances besides colchicine but are nevertheless suitably called c-mitosis and c-tumour reaction, I will hereafter refer to the present reaction as *the camphor reaction* or, abbreviated, *the cf-reaction* even when it is caused by other substances

than camphor and when its type deviates from the typical camphor effect in narrow sense. I will thus discuss cf-forms, cf-cells, cf-colonies, cf-tendencies, cf-activity, and so on.

The common feature in the complex of deviations from the normal cell growth induced by the cf-reaction is the condition that the bud cell does not as normally loosen from the mother cell but grows further still connected with the mother cell. Under the influence of cf-substances, consequently, associations of cells are formed which cannot be brought apart even by heavy shaking of the culture bottle. These associations may in extreme cases grow out into macroscopically visible cf-colonies with thousands of cells (see, for instance, LEVAN and SANDWALL, 1943, the microphoto Fig. 1 *h*). The yeast actually changes into a colony-forming organism.

This hanging together into colonies is no doubt caused by a dissimilarity in the development of the bud cell of the cf-forms as compared with normal budding. This deviation in the formation of the bud cell is striking in extreme cases. Fig. 1 *d*, *f* shows the budding of an untreated cell. The canal between the mother cell and the bud rapidly narrows down into a thin passage. Fig. 1 *i*—*l* pictures the same stages under the influence of camphor. The corresponding canal is often broader and in extreme cases (Fig. 1 *u*, *y*), there is no constriction at all. The new cell is then started as a hypha-like tube, in the interior of which transverse cell-walls may later on develop.

Under advanced narcosis the tube may grow on apically into a long, wide hypha. This may later be divided by cell-walls into an irregular row of cells. Camphor, tertiary butanol and a few other substances cause the development of such formations. Most substances, however, do not narcotize the growth control so deeply. It may be noticed that the tubes make attempts to delimit single cells during their growth. It consequently originates numerous shallow constrictions of the tube, which will show a more or less regular undulation of its outline. Propanol has in my experiments given especially pronounced undulations (Fig. 3: *21*—*23*). The portions of the tubes delimited by the constrictions may later on form separate cells and may even be liberated from the colony. After certain treatments the tube may grow forward under iterated turns to both sides, thus developing a more or less regular zigzag tube or in some cases a clear spiral tube. Such tubes are formed by normal butanol (Fig. 3: *34*—*37*). More irregularly growing cf-tubes may form unilateral swellings, as sometimes observed in pentanol (Fig. 3: *51*—*52*).

Besides the more tube-like cells there may be induced cellular growth in all directions, spherical, vesicular or pumpkin-like cells originating. Often the basic part of these cells is larger, which results in flask- or pear-like formations. Ether and acetone induce this type of growth (Fig. 4 *a—m*). These inflated cells may become very large and may alternate within the cf-colonies with thinner, tube-like cells. In some cases the cf-cells start as tubes, but are soon divided into smaller, rounded cells of a normal cell-shape. Chloroform, ethanol and urethane give such colonies (Fig. 3: *18*, Fig. 4 *y*). They resemble bunches of grapes, and only in the outskirts of the colonies are tube-cells found.

The aromatic substances have in common the feature that the cf-reaction induced by them is hard to demonstrate. There seldom occur the long tubes which are typical of many aliphatic treatments. The cell form of cf-forms of benzene, for instance, is more or less rounded even if it is often tube-like from the beginning (Fig. 2).

It is of some interest to note that at the same time as the yeast changes into a colony-forming organism its individual cells disclose an evident bipolarity. The oldest or basal part of a cell thickens, and from this part only seldom do new tubes grow out. First the apical part of the cell grows on, thereafter new lateral tubes are initiated around the apex of the original cell. These lateral axes of the second order grow out, and from them later on tertiary axes are formed. A growth pattern of a certain regularity may be seen. Its type is similar to those met with in related colony-forming yeast species. Cf-forms of such regular constitution have been found after treatment with chloroform (Fig. 4 *p*) and chloral hydrate (Fig. 4 *r*, *s*). It must be pointed out, however, that this schematic pattern is found only in ideal cases, many irregularities occurring. Tubes may grow out also from the middle or basic part of the cells; the first cell influenced by the cf-reaction may grow out at both ends, resulting in a bilateral colony, or it may form several tubes, resulting in a more radiary colony.

I want to stress the extreme variability in type and the complex nature of the cf-reaction. If it is, as I assume, a narcosis of the normal growth, it may be as complicated as normal growth itself. The control of the many different functions which constitute the normal growth may become switched off in fractions different in different substances. When judging the individual cases it is necessary to take into account the relative position of the activity threshold for cf-action and the toxicity and lethality thresholds.

The c-tumour reaction of root tips makes an interesting parallel to the cf-reaction: in both cases it is a question of a disturbance of the normal cell growth. At the c-tumour reaction, cells which normally grow in length more than breadth will under the influence of narcotics grow more or less isodiametrically. The cf-reaction causes cells that are normally elliptic to grow out into cylinders or tubes. The c-tumour reaction also shows variations in its morphology, depending on which substance has been employed. Colchicine, for instance, brings about small well-defined tumours in *Allium* roots, while acenaphthene gives more undefined, gradually swelling tumours. As a comparison the manifold variations and nuances shown by different narcotics in their action on animals may also be recalled.

2. NUCLEAR MORPHOLOGY.

Owing to the drastic changes in cellular shape and cellular volume induced by the more efficient cf-substances it is to be expected that the nuclear conditions are also disturbed in the cf-cells. BAUCH makes comparisons between the effect of camphor with that of colchicine on higher plants. This is also justified on the basis of his results: he obtained now and then single colonies with larger cells, which afterwards maintained their larger cell-size through many generations. No investigations on the nuclear cytology of the cf-forms were made by BAUCH, however, nor have such studies been made elsewhere.

It may be mentioned, however, that SEGAL (1939) made some efforts to investigate the nuclei of his aberrant yeast cells induced by higher alcohols. He found abnormally shaped nuclei of fixed and HEIDENHAIN-stained material showing a folded or lobated shape (SEGAL, 1939; Fig. 8).

Since it has been shown that cellular divisions go on even after long treatment with cf-substances, it is *a priori* excluded that any permanent or complete colchicine effect can be imposed on yeast by camphor and the other cf-substances. C-mitosis holds up the nuclear divisions and as a consequence also the cellular divisions.

As I considered it a vital point in these investigations to be able to observe directly the behaviour of the nuclei, I have devoted much work on attempts to bring about analysable nuclear stainings in yeast. Using the technique of WINGE and LAUSTSEN (WINGE, 1935, pp. 104—105), I tested different fixatives and different pre-treatments. I found CARNOY, BENDA, FLEMMING, MERKEL, NAVASHIN and ZENKER capable of giving fairly good results. The pre-treatment with sodium hydroxide recom-

mended by WINGE may improve the results considerably. FEULGEN gave the securest results as a stain, but crystal violet and haematoxyline could also give favourable stainings. FEULGEN followed by staining in gentian violet may give quite good contrast, the nuclei being stained red violet while volutine granules and other stainable components of the cell take a pure blue stain.

The fixation and staining both of untreated yeast and of cf-cells are, however, always whimsical. The casual state of the culture and other factors out of control seem to play an important rôle. After a great deal of work it was evident, however, that the yeast nuclei consist of a number of distinct bodies, which go through the mitotic stages in the same manner as chromosomes. Especially at late metaphase individual chromosomes may be visible in side view, their centromeres evidently not yet having been divided. One chromatid is then directed towards each pole, the centromeres seem to have more or less terminal position (Fig. 1 *e*, 2 *i*). The chromosomes at this stage resemble small meiotic bivalents. There is a strong tendency of the chromosomes at all stages to stick together into one or two bodies. The low chromosome number earlier published for yeast (e. g., BADIAN, 1937; SINOTO and YUASA, 1941) may have been influenced by such fusions. After the anaphase separation the two daughter groups of chromosomes tend to be strongly coloured, and each of them may form one or two distinct bodies that often give the impression of a chromosome number of two or four. I have sought very carefully for good metaphase plates, but even in well-stained material most metaphases are not analysable. Often the chromosomes form a ring on a hollow spindle (Fig. 1 *d*). The tendency of the chromatic material to gather in the periphery is found also in resting nuclei (Fig. 2 *c*, *f*).

Not even in the best metaphase plates I have come across (Fig. 1 *a*—*d*) is it possible to give an exact chromosome number. Some facts seem certain, however. The chromosome number is higher than has been generally assumed, ten being a minimum number. Size differences among the chromosomes are often noticed. In some slides two chromosomes seem larger than the rest (Fig. 1 *c*). In other slides, however, very clear plates have been seen without these two large chromosomes. Probably the size of the chromosomes is liable to variations owing to variable action of the pre-treatment in NaOH. The size of the smallest observable chromosomes is at the limit of what is visible in the microscope (about 0,1 μ). It is quite possible that more of the small chromosomes are present than I have been able to demonstrate.

It is also possible that the larger chromosomes are built up from smaller ones sticking together. In the size-class of these chromosomes, and with the rather faint stainings even at their best, the details seen must always be taken with great caution.

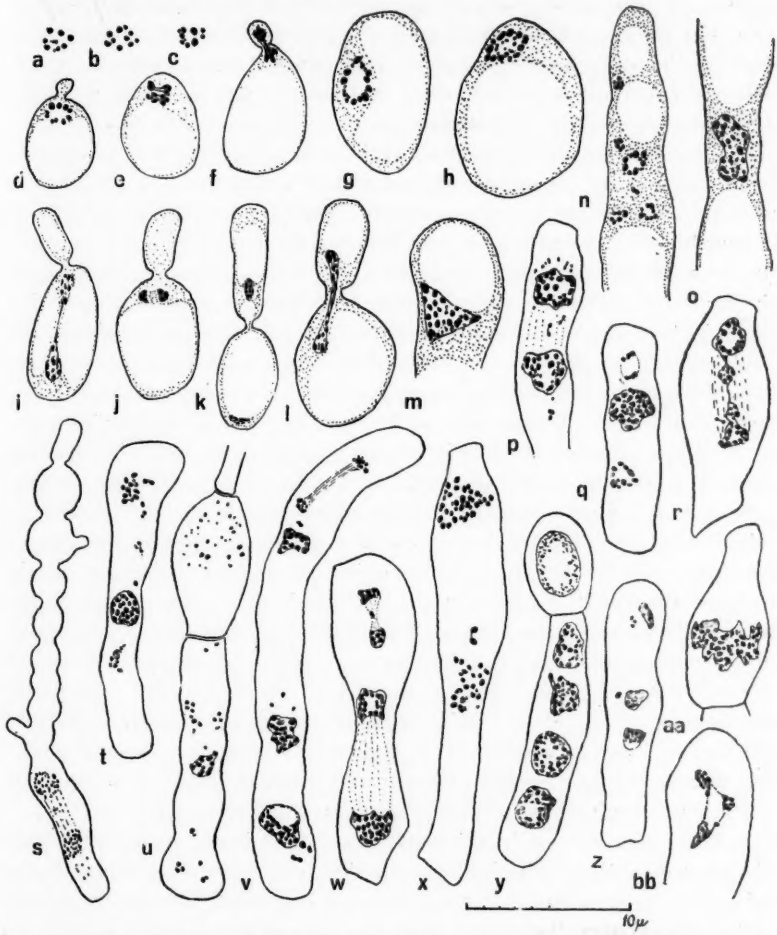

Fig. 1. The caryology of normal yeast and cf-forms. *a—e*: untreated yeast; *f—o*: yeast grown on wort-agar with camphor content; *p—bb*: yeast grown in wort with tertiary butanol; *a—d*: normal metaphases, *e* normal anaphase; *f—l*: various mitotic stages in giant cells; *g—h*: metaphases with doubled chromosome number; *m—bb*: various nuclear disturbances found during advanced cf-growth; *a—o*: FEULGEN, *p—bb*: acetocarmine. — × 2500 (*s*: × 1200).

The nuclear conditions of the cf-forms were first studied on yeast grown on agar prepared from wort solutions of known camphor content. The outgrowing giant colonies were smeared on slides and fixed and stained in the same way as untreated yeast. In the giant colonies thus produced the majority of cells are always found to be of the normal type, but they are intermingled with a certain frequency of cf-cells. Most of the cells have normal nuclear conditions. About the same chromatin amount is present as in untreated material. The separate chromosomes may be estimated to ten in the camphor-treated cells also.

After such treatment, however, it is noticed that the cell-size varies more than usual even among the normally formed cells. I found that some of the large cells had larger nuclei than normally, and in some favourably fixed giant cells it was possible to demonstrate the doubled chromosome number of at least 20 (Fig. 1 *g*, *h*). These are evidently chromosome-doubled cells, and the chromosome doublings are independent of the tube-like cf-growth. Such cells look quite viable; no doubt they may give rise to tetraploid strains if isolated. In the cell population of the giant colony, however, the tetraploid cells are in such low frequency as to make it very improbable that they can stand the competition with normal diploid cells. The mechanism of their doubling cannot be known with certainty. The tetraploid cells have normal mitosis with full spindle activity. If they have been induced by c-mitosis, this must have been transient, only affecting one mitotic cycle. Since it is known that the yeast has the ability of rapidly acquiring a certain resistance towards cf-treatments, especially if the concentration of the active substance is immediately above the threshold value, it is perhaps not absolutely inconceivable that one mitosis may become narcotized, but that the cell after that again takes command over its mitotic mechanism, the subsequent mitoses being normal. The other possibility for the origin of tetraploidy is an endomitotic process. Unfortunately, the nature of the material impedes any close study of the details of these processes, so a decision on this point must rest so far.

The main interest in this material centres in the real outgrowing cf-forms. In the beginning, during the first week of treatment, the mitoses of even extreme cf-cells are seen to proceed normally, spindle disturbances being exceptions. In many cases it was possible to estimate the chromosome number within the cf-forms. Usually it was normal diploid. Often it occurred that the nuclei of the cf-cells were more diffuse than neighbouring normal cells, they took the stain more faintly and, generally speaking, showed indications of being in a deeper resting

condition than the other surrounding cells. This may suggest the possibility of an endomitotic mechanism. When the chromosomes are again stainable, there is often found in the more advanced cf-forms a higher chromosome number than normally, 4x, 8x or still higher. Usually the spindles are functioning normally. Only in a few cases (Fig. 1 *m—o*) were there found indications of clear spindle disturbances, such as several nuclei lying close together and clearly showing variable chromosome number.

In the autumn of 1943 I made a number of acetocarmine slides of yeast grown 2 or 3 weeks on wort with different cf-substances. These slides contained a great frequency of much more advanced cf-forms than the above FEULGEN-slides. The acetocarmine slides were stored, which could be done because the acetocarmine employed contained glycerol. In 1946 I re-investigated these slides, finding, as might have been expected, that the cells had taken so much stain as to be quite blackish-purple. In some of them, however, I faintly saw some darkly stained bodies resembling nuclei. I accordingly put the slides in 45 % acetic acid in order to wash out the excess of stain. Thereafter the slides were made permanent by mounting in canada balsam. It was found that the cell form had remained almost unchanged during this procedure. In some of the slides fairly good nuclear stainings were present. Especially one slide, treated a fortnight with 0,2 mol/l tertiary butanol, showed excellent staining. In this slide I learned more about the chromosome conditions of the cf-forms than in the several hundreds of earlier FEULGEN-slides, although, broadly speaking, the nuclear disturbances met with in this acetocarmine slide agreed with those earlier studied. They were, however, more frequent and more extreme.

The chromosomes were undoubtedly somewhat more swollen than after ordinary fixation and staining, which may not be entirely disadvantageous considering the small size of the chromosomes. The cells were also decidedly larger than after the other fixation methods. I found that I had interpreted correctly the chromosome form and size in the earlier slides. Here there were often found single chromosomes lying free in the plasm and furnishing a good opportunity to study the chromosome shape and size. Almost all cells in this slide represented very extreme cf-disturbances. The nuclear conditions were highly irregular, instances of cells from this slide being pictured in Fig. 1 *p—bb*. It was noticed that the synchronization between cellular growth and mitosis was often disturbed. Thus, in the periphery of the colonies

there were very often no nuclei present, although the constrictions of the cell-wall indicating the limits of the future cells were fully prepared. The mitotic activity was evidently lagging behind the cellular growth (Fig. 1 *s*). In the more centrally located cells, on the other hand, it was not unusual with four or more large nuclei in one cell (Fig. 1 *y*). The nuclear size was highly varying. Often two very large nuclei with many chromosomes in each and two decidedly smaller nuclei were present in one cell (Fig. 1 *w*). In this case it could be inferred from the mutual positions of the nuclei and from spindle fibres remaining between each pair of nuclei that the larger two represented telophase of one mitosis and the two smaller ones of another mitosis. The distance between the telophase nuclei was in this and in other cases roughly proportionate to their size, the larger nuclei lying decidedly more apart than the smaller ones. The spindle has perhaps a certain size relation to the number of chromosomes participating in a mitosis. Ordinarily all spindles were located in the longitudinal direction of the tube cells, but very small nuclei could develop their spindles transversally.

It has a certain interest here to be able to demonstrate that even at such an advanced stage of cf-growth the spindles function and do so rather regularly. The anaphases, it is true, all showed serious disturbances, lagging chromosomes, multipolarity, formation of restitution nuclei, and so on. Sometimes the entire nuclear material was scattered out over the whole cell (Fig. 1 *u*, the upper cell). In this case a decided tendency to an arrangement of the chromosomes in pairs could be seen. This cell might represent a c-metaphase, and the pairs may be c-pairs.

Since certain objections may be raised against the use of acetocarmine in the staining of yeast chromosomes, this stain not being so selective a nuclear stain as desirable, I decided to repeat the stainings of similarly treated cultures by the FEULGEN technique. In the summer of 1946 some cultures were put in various concentrations of tertiary butanol and of benzene. Samples of the cultures were centrifuged every second day during the treatment. FEULGEN slides were prepared from the sedimented cells. The slides of both types of treatment showed normal mitoses during the first ten days mostly, although the typical cf-forms occurred as usual. Not until the 12th and 14th day did distinct nuclear disturbances begin to be frequent. Especially some fixations from the 16th day of a culture treated with a strong benzene concentration (0,012 mol/l) gave an abundance of mitotic disturbances, allowing an analysis of the nuclear behaviour during the cf-growth. (Here as elsewhere in the present paper »the first day» refers to ob-

servations 20—24 hours after the beginning of the treatment, »the second day» means a treatment of two days, and so on.)

Fig. 2 shows some cells from this treatment. A microphoto of a

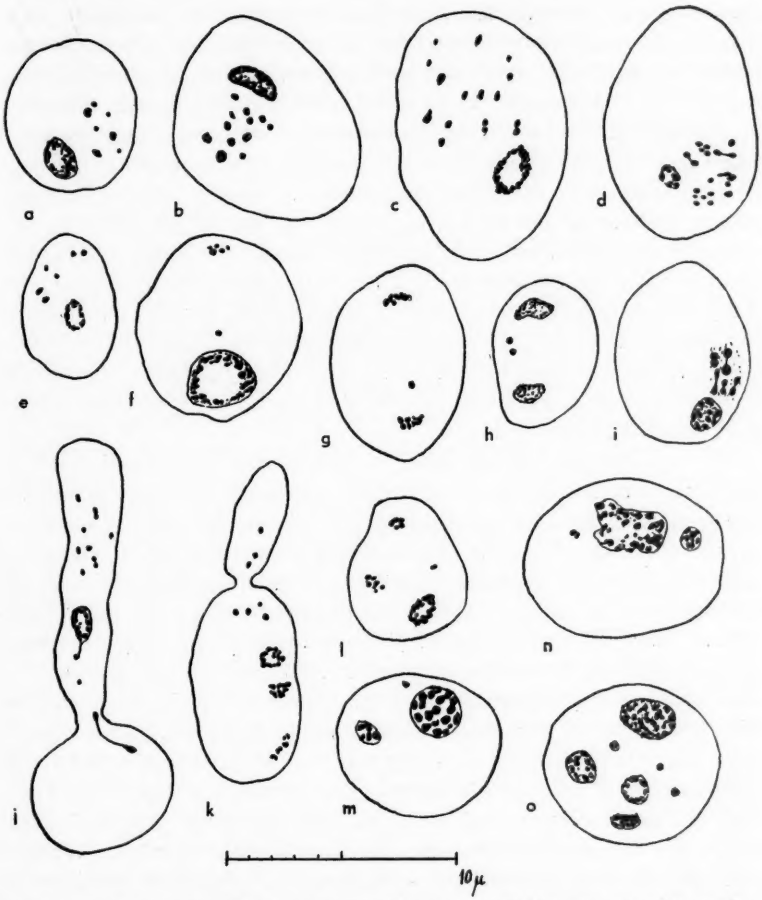

Fig. 2. Yeast cells treated for 16 days with 0,012 mol benzene, showing various mitotic disturbances, chromosomes scattered outside the nucleus (*a—e*, *j—k*), vagabond chromosomes (*f—h*), accessory spindle (*i*), multipolar telophase (*l—o*); FEULGEN. — × 3500.

view field from a similar treatment has already been published in a preliminary communication to Nature (LEVAN, 1946). In this micro-

photo some of the commonest types of irregularities were gathered. It is seen from the present Fig. 2 that exactly the same type of disturbances are found here as in the aceto-carmine slide described above. The control in FEULGEN can thus be said to have strengthened the conclusions drawn above. Also here there occur nuclei of dissimilar size, some being many times the size of untreated nuclei. Often solitary bodies, strongly suggestive of chromosomes, are scattered outside the nuclei. What increases the impression of chromosomes is the often occurring clear doubleness of the bodies: it looks as if single chromosomes had divided in their abnormal position. In some cases two groups of chromosomes or two nuclei are situated one at each end of a cell with one single or one double body lying between them (Fig. 2 *g*, *h*), evidently laggards from the foregoing mitosis. Although the spindle structures are not stained by this technique, differences in refraction in some cases gave clear evidence of spindles. In Fig. 2 *i*, for instance, one spindle with five dividing chromosomes was observed outside a nucleus. Pictures such as Fig. 2 *l*, *o* are strikingly similar to multipolar telophases, such as are often seen in higher plants, for instance after a weak c-mitotic influence.

As already pointed out above, a special interest is attached to the solitary chromatic bodies lying outside the denser clusters of nuclear material. Benzene acts upsetting on the mitotic spindles, which under normal conditions keep the chromosomes crowded together. The result is that the chromosomes are scattered out, allowing the study of single chromosomes as well as of the two daughter halves of one chromosome. Thus, an opportunity is given, which seldom occurs normally in yeast, to observe single chromosomes. It appears that the chromosome size is decidedly smaller than has been assumed by those workers who have given a low chromosome number for yeast. The chromosome size established by studying this treated material is, instead, in good agreement with the size of the smallest single bodies found in the best fixations of untreated material. As mentioned above, in such material a chromosome number of at least ten was counted. While in this untreated material there was always a suspicion, even in the clearest cases, that these bodies were not entirely free from each other but might be chromomeres belonging to larger chromosomes, this benzene-treated material clearly shows that they really are free chromosomes, which may be present entirely separated from each other. The only other alternative is in my opinion that the treatment has effected

some fragmentation of chromosomes. This alternative seems less likely considering the regularity in the arrangement of the free bodies.

Summing-up the results of the caryological analysis of the cf-forms, it may be stated that the drastic changes in cellular shape induced by the cf-substances are also accompanied by considerable deviations from normal conditions in mitosis. There has been observed a highly increased chromosome number of individual nuclei. Mitotic disturbances, although rare in the beginning, later on occur regularly. Whether the increase observed in chromosome number is brought about by a transient c-mitosis or by an endomitotic process has not been decided.

III. THE ALIPHATIC ALCOHOLS.

In this and following chapters (III—VI) a systematic account is given of the experiments performed with the various substances. When possible the threshold values of lethality, toxicity and cf-reaction are recorded at the end of the description of each substance. Characteristic morphological features in the development of the cf-forms are also mentioned. Each group of substances is concluded with a brief general discussion.

1. METHANOL.

a. *Orientating experiment* ($^{8}/_{9}$, 43). — Concentrations: 0,01—2 mol/l. After 2 days the cells were still living in 2 mol, but no development occurred. In 1 and 0,5 mol almost exclusively cf-growth occurred after 20 hours, on the 2nd day 1 mol still held many cf-forms, but in 0,5 mol most cells were now normal. Weak tendencies to cf-reaction were perceived in 0,2 and 0,1 mol. All except 2 mol were in fermentation on the 2nd day.

b. *New series* ($^{26}/_{1}$, 44). — Concentrations: 0,1—2 mol. In this experiment true cf-forms were found only in 2 mol. Tendencies to cf-growth were also seen in 1 mol. 2 mol was not lethal, but the fermentation was very much retarded. On the 17th day this concentration was fermenting, however.

The cf-forms of this alcohol were not extreme (Fig. 3: *1—14*). On the 2nd day the cells of the concentration 2 mol were narrow and oblongate. The buds were often tube-like. Later on the cells were often rounded off. The number of cells in each cf-colony was never especially high. In one count the colonies contained 2—10 cells

(Table 1). The outgrowing tubes often had a somewhat undulated outline (Fig. 3: 7, *8*).

Tested zone: 0,01—2 mol
Lethality: None
Toxicity: 2 mol
Cf-reaction: (0,5—1)—2 mol.

2. ETHANOL.

a. *Orientating experiment* ($^{8}/_{9}$, 43). — Concentrations: 0,01—2 mol. In 2 mol the cells did not show any development during the experiment, this culture never coming into fermentation. 1 mol showed cf-growth, after 2 days rather large cf-colonies of 20—30 cells were seen. 0,5 and 0,2 mol also gave tendencies to cf-growth.

b. *New series* ($^{26}/_{1}$, 44). — Concentrations: 0,1—2 mol. The results were similar to the former ones, but the frequency of cf-forms was lower. The only concentration with any considerable frequency of cf-forms was 1 mol after 20 hours. After 2 days this culture had also passed over into a phase showing preponderantly normal cells. All except 2 mol came into fermentation in 2 days. 2 mol did not show fermentation during the entire experimental period, which was 15 days.

c. *Testing of the zone 1—2 mol* ($^{31}/_{1}$, 44). — Concentrations: 6, 7, 8, 9, 10, 11, 12 volume % (1,0—2,0 mol). In this series cf-forms were observed in the following treatments:

6 % after 20 hours and 2 days
7 » » 20 » , 2, 3 and 7 days
8 » » 20 » , 2, 3 » 7 »
9 » » 7 and 12 days

6—8 % thus gave cf-forms in the beginning of the experiment, 9 % gave cf-forms later on. 10—12 % showed no development during the 15 days of the experiment.

The general type of the cf-forms was similar to that of methanol (Fig. 3: *15—18*). One long tube-like bud grows out without separating from the mother cell. Often this tube from the beginning or gradually develops a great many constrictions (Fig. 3: *16*, *17*), later on delimiting separate cells. In that way long rows of cells arise.

Especially in the higher concentrations a long period of treatment, 7 days or more, brings about the origin of large colonies of the grape-cluster type (Fig. 3: *18*).

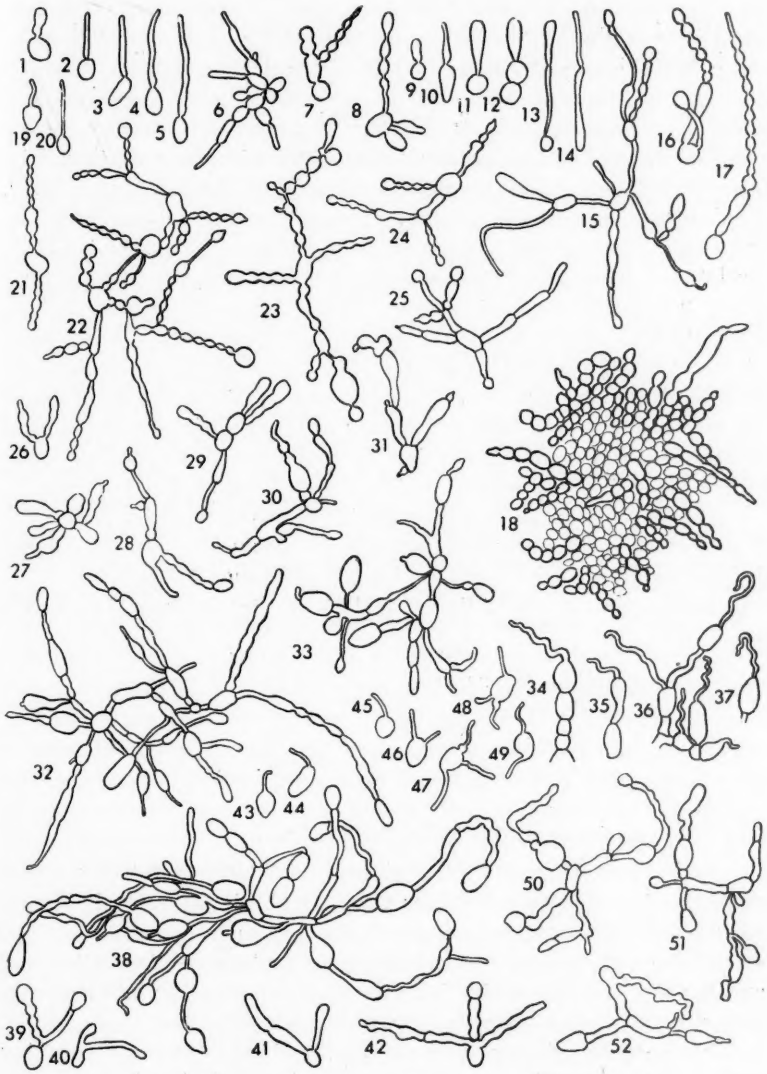

Fig. 3. Outline drawings of cf-forms induced by aliphatic alcohols: methanol (*1—14*), ethanol (*15—18*), propanol (*19—25*), normal butanol (*26—37*), iso-butanol (*38*), tertiary butanol (*39—42*), iso-pentanol (*43—52*). — × 400.

Tested zone:	0,01—2 mol	Toxicity:	1—1,7 mol
Lethality:	1,7 —2 »	Cf-reaction:	1—1,5 »

3. PROPANOL.

Both normal and iso-propanol were tested with concentration series from 0,02 to 2 mol ($^{21}/_{1}$, 44). Both showed little development in 1—2 mol. The iso-form seems to be a little less toxic than the normal form. In the former one cf-forms started growing out after 2 days in 1 mol, in the latter one no growth occurred in this concentration until after 7 days. 0,5 mol of the iso-form allowed full fermentation after 2 days, while 0,5 mol of the normal form gave no fermentation in 15 days. At that time it was not dead, however, but contained a decided cf-growth. In the normal form 0,2 mol is the highest concentration allowing fermentation in 2 days. A similar difference between the normal and the iso-form is apparent in the cf-reaction also. Cf-forms are found in 0,1—0,5 mol of the normal and in 0,2—0,5 mol of the iso-form.

The appearance of the cf-forms is characteristic (Fig. 3: *19—25*). A maximum in the aliphatic series is here reached concerning the undulated outlines of the cf-cells. Complicated such types were found, for instance, in 0,5 mol of the normal form after 16 days (Fig. 3: 22).

	Normal	Iso
Tested zone:	0,02—2	0,02—2 mol
Lethality:	1 —2	2 »
Toxicity:	0,5	0,5 —1 »
Cf-reaction:	0,1 —0,5	0,2 —0,5 »

4. BUTANOL, C_4H_9OH.

a. *Normal, primary* (*$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2OH$*), *orientating experiment* ($^{8}/_{9}$, 43). — Concentrations: 0,01—2 mol. In 0,5—2 mol the cells were killed. Cf-growth occurred in 0,05—0,2 mol. Thus, 0,1 showed after 2 days 100 % cf-forms, while 0,2 mol had only little development and 0,05 mol had predominatingly normal growth. The latter concentration had 100 % cf-growth after 20 hours.

b. *Normal, primary, new series* ($^{28}/_{1}$, 44). — Broadly speaking, similar results: 0,2 still had very little development after 6 days; probably most cells were killed. Cf-forms were found in 0,01—0,1 mol.

The cf-forms of this alcohol (Fig. 3: *26—37*) were often large and contained irregularly shaped cells. Their general type is more extreme

than in lower alcohols. Large, rounded, oviform or piriform cells are formed, from which long hyphal threads take their origin. The distal tubes are not seldom turned in spirals, which may sometimes be surprisingly equal (Fig. 3: *34—37*). If the origin of bead-like tubes indicates a series of intermittent impulses to normal growth, the spiral structure may indicate a more continuously acting asymmetric impulse.

c. *Normal, secondary butanol* ($CH_3 \cdot CH_2 \cdot CHOH \cdot CH_3$) ($^{19}/_6$, 45). — Concentrations: 0,01—0,5 mol. While the cells in 0,5 mol did not show any development, 0,2 mol was in full fermentation after 2 days. Only 0,2 mol had any considerable number of cf-forms.

d. *Iso-butanol* [$(CH_3)_2 \cdot CH \cdot CH_2OH$] ($^{28}/_1$, 44). — Concentrations: 0,01—2 mol. Conditions resembled those of normal butanol (above, b): 0,2—2 mol showed lethality. The toxic effect may have been somewhat less than in the normal form. Cf-forms occurred in 0,02—0,1 mol.

The cf-forms had the same type as in normal butanol, or were even somewhat more irregular (Fig. 3: *38*). In the same colony large, inflated cells alternated with long thin tubes. It occurred that the tube-cell grew out from the middle of the mother-cell and not distally as usual. Sometimes two tubes grew out from the same end of one cell, as was observed to happen also in normal butanol (Fig. 3: *26*). These V-shaped cf-forms are still more common in tertiary butanol (Fig. 3: *39—42*).

e. *Tertiary butanol* [$(CH_3)_3 \cdot COH$], *orientating experiment* ($^8/_9$, 43). — Concentrations: 0,01—2 mol. The cells were killed in 1—2 mol. 0,5 mol gave poor development, but small cf-tubes grew out in 2 days. They were narrow and twisted, and the cells showed poor viability. In 0,1—0,2 mol there was 100 % cf-growth after 20 hours. 0,1 mol already returned to mostly normal growth after 2 days. Single cf-forms also occurred now and then in 0,05 mol.

f. *Tertiary butanol, new series* ($^{28}/_1$, 44). — Same concentrations and, broadly speaking, same results as the foregoing. Cf-forms occurred in 0,02—0,5 mol, especially numerous and well-developed cf-forms were seen in 0,1—0,2 mol.

The cf-growth of this alcohol is typical in appearance as well as in completeness. The long, thin hyphae hang together in large colonies in which the cells form an airy and porous tissue. The colonies often reach a size that they may be seen macroscopically as cotton-like lumps swimming in the culture fluid. Among all the alcohols studied this is the one which most easily gives cf-growth. Its active zone is unusually

wide, which is connected with the rather high position of the lethality limit.

	Normal primary	Normal secondary	Iso	Tertiary
Tested zone:	0,01—2	0,01—0,5	0,01—2	0,01—2 mol
Lethality:	0,2 —2	0,5	0,2 —2	1 —2 »
Toxicity:	0,05—0,1	No	0,05—0,1	0,1 —0,5 »
Cf-reaction:	0,01—0,1	0,1 —0,2	0,02—0,1	0,05—0,5 »

5. PENTANOL, $C_5H_{11}OH$.

a. *Iso-pentanol* ($^{20}/_1$, 44). — Concentrations: 0,005—0,5 mol. Above 0,1 mol lethality. Cf-growth occurs in (0,005)—0,01—0,05 mol. Numerous but little developed cf-forms were found in 0,05 mol after 20 hours. At the same time 0,02 mol had preponderantly normal cells; only 10 % cf-forms were seen. This concentration was the highest one giving full fermentation in 2 days. 0,05 mol did not get into fermentation until after 17 days. After 2 days 0,02 mol contained many complicated cf-colonies. These fell apart later on, so after 5 days only few-celled cf-forms were met with, and after 17 days most of the cells were normal.

The cf-forms of pentanol were characteristic. In the strongest concentration, 0,05 mol, they were little developed (Fig. 3: *43—49*). They consisted of one rounded cell with one to three narrow tubes. Sometimes the tube made an effort to round itself off into a more normal bud cell, often evidently without success. It then continued growing out into a thin tube, the proximal, basic part of which showed a spherical swelling (Fig. 3: *47*, *48*). In lower concentrations (0,02 mol) a special type of cf-forms occurred having broad, somewhat indented cells with irregular outline (Fig. 3: *50—52*). Certain cells of these cf-forms could form wide, curved tubes with small irregular humps, the whole cell being reminiscent of a maple seed. This type occurred in two separate experiments with iso-pentanol, but has scarcely been observed elsewhere, so it may be characteristic of this alcohol.

b. *Tertiary pentanol* [*Amylene hydrate*, $(CH_3)_2 \cdot C(OH) \cdot CH_2 \cdot CH_3$] ($^{11}/_8$, 43). — Only very preliminary results are available from this substance. After a test in a moist chamber resulting in positive cf-reaction one series of concentrations between 0,001 and 2 mol was arranged. After 20 hours 0,5—2 mol had killed the cells, 0,1—0,2 mol showed cf-growth, 0,2 mol in a high degree (87 %).

	Iso	Tertiary
Tested zone:	0,005—0,5	0,001—2 mol
Lethality:	0,1 —0,5	0,5 —2 »
Toxicity:	0,02 —0,05	0,2 —0,5 »
Cf-reaction:	0,01 —0,05	(0,1)—0,2 —0,5 »

6. OCTANOL, $C_8H_{17}OH$.

a. *Orientating experiment* ($^8/_9$, 43). — Concentrations: 0,001—0,2 mol. 0,05—0,2 mol had a killing action. In 0,02 there occurred little development, but the cells showed clear cf-tendencies before dying. Typical cf-forms were found in 0,005—0,01 mol. Even in 0,002 clear cf-tendencies were perceivable after 24 hours.

b. *New series* ($^{30}/_1$, 44). — Concentrations: 0,001—0,05 mol. The results were in agreement with the preceding series, but in this series 0,005 mol was almost lethal, cf-forms being found only in 0,002 mol and single ones in 0,001 mol. The cf-forms are less developed in this alcohol than in the former ones. Usually they consist only of one cell with an outgrowing tube, larger colonies not being formed. It is evident that the threshold of cf-growth lies rather close to the toxicity threshold, which has the result that any decided cf-growth can occur only in one concentration step without almost total lethality.

Tested zone: 0,001—0,05 mol
Lethality: (0,005)—0,01—0,05 mol
Toxicity: 0,002—0,005 mol
Cf-reaction: 0,002—0,005—(0,01) mol.

7. LAURYL ALCOHOL (DODECANOL, $C_{12}H_{25}OH$).

Of this as of the following alcohols only qualitative tests were made with saturated solutions. Five cultures were put within the saturated zone with different excess of undissolved substance. The quantity of substance in these cultures corresponded to 0,001—0,01 mol. In none of these tests did total lethality occur. The often encountered phenomenon that the lethality percentage increases with increasing excess of precipitate in saturated solution is very clear:

Excess corresponding to a concentration of:	0,001	0,002	0,01 mol
Lethality %	38,7	84,4	88,2
Fermentation after 2 days	++	+	—

In none of the cultures were any typical cf-forms found, although a general tendency to elongated cell-growth was common.

8. HIGHER ALCOHOLS.

a. *Cetyl alcohol* (*Hexadecanol*, $C_{16}H_{33}OH$). — Only one solution was tested, viz. saturated solution with excess of precipitate. This proved perfectly harmless to the yeast cells, which showed a lethality of only 6 % and entered into full fermentation in 2 days. The tendencies to cf-growth were somewhat more definite than in the preceding. No more developed cf-colonies were found, however, although simple involution forms were common.

b. *Octadecanol*, $C_{18}H_{37}OH$. — One saturated solution with excess of precipitate was tested. Lethality: 2,2 %, full fermentation in 2 days. Like the preceding one it showed a certain tendency to cf-growth, although less pronounced than this.

9. SURVEY OF THE ALIPHATIC ALCOHOLS.

Certain interesting points are apparent on surveying the experimental results of the different alcohols investigated. As is well familiar from many experiments and is stated in the so-called rule of the homologous series of RICHARDSON, the activity of the alcohols increases with increasing number of C-atoms. This has been pointed out for the alcohols several times, for instance, by SEGAL (l. c.) concerning the alcohols tested by him. Even more than the number of C-atoms, however, the water solubility of the alcohols gives a picture of their toxicity (RICHET's rule). Lethal concentrations of the different alcohols met with in the present experiments may be roughly correlated with the solubilities in the following manner:

Solubility	Lowest lethal concentration	Number of alcohols tested
∞	1	5
1	0,2	3
0,3	0,1	1
0,01	0,005	1
$< 0{,}001$	no lethality	3

The lethality threshold sinks up to a solubility of 0,01. Below that there appears the well-known »cut-off» in toxicity, which depends on the lethality threshold lying above the solubility; concentrations strong enough to act lethally cannot be attained (cf. FERGUSON, 1939).

Another measure of the toxicity may be had from an estimation of the time period necessary for a culture to reach full fermentation. The control cultures, normally, were fermenting after 2 days. Below

are given the highest concentrations of each treatment allowing full fermentation in 2 days; in these cultures consequently no significant toxicity can be present:

Methanol	1	mol
Ethanol	1	»
Iso-propanol	0,5	»
Normal propanol	0,2	»
Tertiary butanol	0,2	»
Normal and iso-butanol	0,05	»
Iso-pentanol	0,02	»
Octanol	0,002	»
Lauryl, cetyl and octadecyl alcohol	Saturated solution	

This estimation of the fermentation is, of course, rather inexact. It would have been preferable to determine the fermentation intensity by quantitative methods. Exact cell counts for determining the rapidity of growth of the cultures were undertaken and might have been used for measuring the toxicity. Since the present experiments are largely of an explorative nature, however, I prefer not to press the results too hard. For the present a rough estimate of the main lines seems to be sufficient. In determining the toxic zones in the preceding paragraphs, the decrease in cell number in comparison with the controls was taken into account for each tested substance.

The main interest of this investigation has been directed to the cf-reaction. In Table 1 a record has been made of the concentrations of the different alcohols which have given any certain frequency of cf-forms after 20 hours. It is seen that all alcohols have shown at least tendencies to cf-growth. They certainly all possess activity, though their efficiency is highly variable (the terms »activity» and »efficiency» are used in the same meaning as in ÖSTERGREN, 1944, pp. 434—435). The correlation between cf-activity and water solubility is evident.

The efficiency of a substance for inducing a certain reaction may be judged in different ways. The effective concentration zone, measured as the number of concentrations in a certain dilution series which have given the reaction, is one measure. The maximal frequency of cf-forms attained in a substance is another. The number of cells present in the individual cf-colonies is a third measure. These measures may be found in Table 1, and it is seen that they are correlated to each other. Not taking into account the different isomeres of each alcohol, the following is found:

TABLE 1. *The cf-reaction of the aliphatic alcohols.*

Substance		Molar concentration	Number of cells per cf-colony																		Total number of colonies	Average number of cells per cf-form	Percentage of cf-forms	Lethality percentage	Number of days to full fermentation
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18					
Methanol		2	—	5	4	12	2	8	—	2	—	1	—	—	—	—	—	—	—	—	34	4,5	60,7	30,0	17
Ethanol		1,4	1	3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	2,3	60,0	77,8	7
		1,2	—	8	—	5	1	1	—	—	—	—	—	—	—	—	—	—	—	—	15	3,1	42,9	56,3	5
		1,0	—	1	5	4	2	—	2	—	—	—	—	—	—	—	—	—	—	—	14	4,1	43,7	52,2	2
Propanol	Normal	0,5	—	18	4	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	26	2,5	92,9	39,1	17
		0,2	—	4	2	—	1	2	1	—	1	—	—	—	—	—	—	—	—	—	11	4,3	5,4	1,0	2
	Iso	0,5	2	17	6	2	5	4	9	3	3	—	1	1	1	—	—	—	—	1	55	5,1	56,7	3,0	2
		0,2	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	3,0	0,7	3,1	2
Butanol	Normal	0,1	—	3	1	1	1	2	—	2	—	—	—	—	—	—	—	—	—	—	10	4,6	76,9	0	15
		0,05	—	3	1	2	1	2	3	2	2	1	2	—	1	—	—	—	—	—	20	6,7	20,4	0,7	2
		0,02	—	2	—	2	—	—	1	1	—	1	—	1	1	1	—	—	—	—	10	7,6	4,7	0,9	2
	Iso	0,1	—	2	1	7	—	1	—	3	1	—	—	—	—	—	—	—	—	—	15	4,9	100,0	6,3	6
		0,05	1	10	1	18	1	1	1	1	2	1	1	—	—	—	2	—	—	—	40	4,8	51,2	2,5	2
		0,02	—	—	—	—	—	1	3	—	—	2	—	—	—	—	—	—	—	—	6	7,8	2,3	0,3	2
	Tertiary	0,3	—	5	2	3	4	2	1	3	—	—	—	1	—	—	—	—	—	—	21	4,9	100,0	61,9	4
		0,2	—	1	1	3	1	—	2	1	1	—	—	—	—	—	—	—	—	—	10	5,3	100,0	16,7	2
		0,1	1	16	1	8	1	4	1	1	3	2	1	—	—	—	1	—	—	—	40	4,6	90,9	2,2	2
		0,05	—	1	—	2	—	1	—	—	1	—	—	—	—	—	—	—	—	—	5	5,0	3,7	1,5	2
		0,02	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	2	6,5	0,9	0,4	2
Pentanol	Iso	0,05	6	5	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	15	2,1	100,0	73,3	17
		0,02	—	5	—	4	3	5	4	3	2	—	—	—	—	—	—	—	—	—	26	5,4	10,6	4,7	2
		0,01	—	3	2	4	1	2	2	2	—	—	—	—	—	—	—	—	—	—	16	4,7	4,1	4,6	2
		0,005	—	1	2	—	1	—	—	—	—	—	—	—	—	—	—	1	—	—	5	5,8	1,5	1,5	2
	Tertiary	0,2	—	18	12	21	7	10	4	5	1	—	—	—	—	—	—	—	—	—	78	4,2	86,7	12,7	?
Octanol		0,002	1	6	3	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	14	2,9	4,3	19,5	2
		0,001	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	4,0	0,2	4,7	2

Alcohol	Number of concentrations with cf-forms	Maximal percentage of cf-forms	Average cell number in each cf-colony
Methanol	1	60,7	4,5
Ethanol	1,5	60,0	3,4
Propanol	2	92,9	3,7
Butanol	3,7	100,0	5,8
Pentanol	4	100,0	4,4
Octanol	2	4,3	3,5

A fourth criterion of the efficiency of a substance may be had by studying the cell-shape within the cf-colonies. Cf-colonies of long hyphae with parallel or irregular outlines must be considered more deeply narcotized than such as have undulated walls or such as have tubes alternating with normal cells.

It will be seen that all these criteria point to a maximum of efficiency at 4 to 5 C-atoms: 4 concentrations give effect, 100 % of the cells may turn into cf-forms, the average number of cells in single cf-colonies was about 5, the cell-shape of the cf-forms in butanol and pentanol is extremely elongated. Tertiary butanol even exceeds camphor in this respect.

IV. OTHER ALIPHATIC SUBSTANCES.

1. ETHYL ETHER.

a. *Orientating experiment* ($^8/_9$, 43). — Concentrations: 0,01—2 mol. The concentrations, however, are probably somewhat inexact, since the experiment was performed in test tubes closed with corks. The evaporation of the ether may not have been effectively checked. Killing concentrations: 1—2 mol; cf-growth occurred in 0,1—0,5 mol, in 0,5 almost to 100 %.

b. *New series* ($^2/_2$, 44). — Concentrations: 2, 3, 4, 5, 6, 7 volume % (about 0,2—0,7 mol). This experiment was made in soda water bottles with air-proof stoppers. All except 2 % showed a high degree of lethality after 20 hours, 2 % had a lethality of 48 %. This concentration started to ferment weakly after 2 days. After 6 days 4—7 % were all dead, 3 % showed no development but contained living cells, 2 % had come into lively fermentation.

Cf-forms were found occasionally in the lethal concentrations, a certain cf-growth having started before death. Only 2 % showed any

high degree of cf-growth: after 1 and 2 days it held almost 100 % cf-forms. The type of cf-forms (Fig. 4 *a—f*) was consistent in both experiments. Both long hyphae and large bottle-shaped or vesicular cells were formed. These latter may swell up into a considerable volume (Fig. 4 *d, f*), exceeding that of the cf-cells of the aliphatic alcohols.

Tested zone: 0,01—2 mol
Lethality: 0,4 —2 »
Toxicity: 0,2—0,4 »
Cf-reaction: 0,1—0,3 »

2. ACETONE.

a. *Orientating experiment* ($^8/_9$, 43). — Concentrations: 0,01—2 mol. Lethality: 2 mol. In 1 mol the development is checked, but after 2 days living cells are still found. Cf-forms are found in 0,2—0,5 mol. In 0,1 mol tendencies to cf-growth are found, but the cells are preponderantly normal. After 2 days large and complicated cf-forms were found in 0,5 mol.

b. *New series* ($^2/_2$, 44). — Concentrations: 1, 2, 3, 4, 5 volume % (0,14—0,68 mol). 5 % showed no development, 4 % gave weak fermentation after 5 days and then contained quite large cf-forms, 3 % gave little development in 2 days, most cells showing cf-growth. In five days this concentration was in full fermentation, and it then contained mostly normal cells. 1 and 2 % fermented already after 2 days. These two contained 2,7 % and 58,7 % cf-forms respectively after 20 hours.

The cf-forms of acetone have a characteristic type (Fig. 4 *g—n*) resembling the cf-forms of ether. Quite long tubes are formed, which often begin to swell up into large pumpkin- or pear-like formations. These large cells may often take an irregular, indented outline (Fig. 4 *m*) directly indicating lacking growth control. The largest cells often take especially strong staining in aceto-carmine, which may point to sublethality.

Tested zone: 0,01—2 mol
Lethality: 2 »
Toxicity: 0,4 —1 »
Cf-reaction: 0,2 —0,5 »

3. CHLOROFORM.

a. *Orientating experiment* ($^8/_9$, 43). — Concentrations: 0,0005—0,1 mol. Lethality: 0,05—0,1. 0,05 gave some tendencies to cf-growth before dying. 0,02 gave a considerable frequency of cf-forms after 20

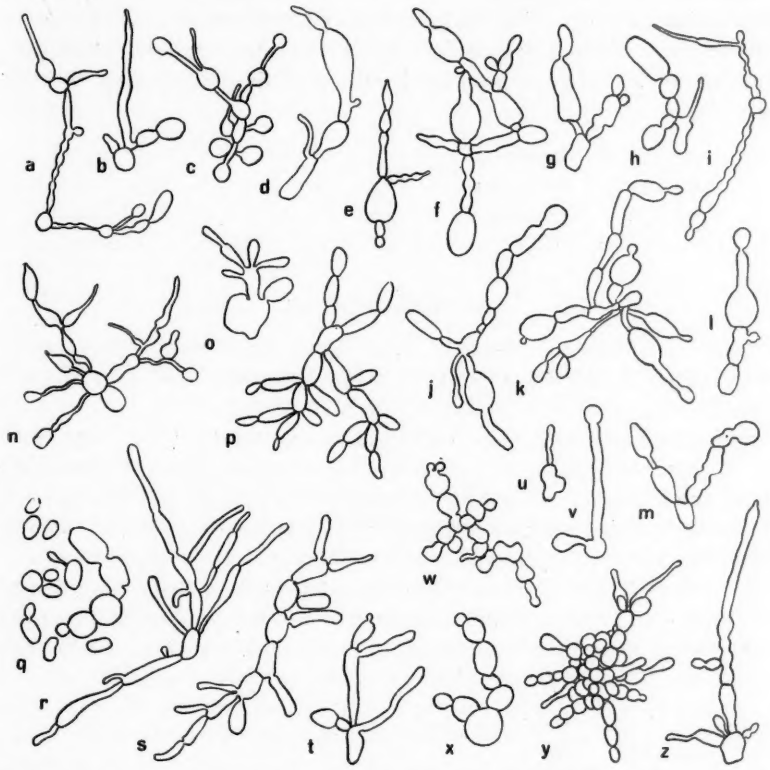

Fig. 4. Outline drawings of cf-forms induced by various aliphatic substances: ethyl ether (*a—f*), acetone (*g—n*), chloroform (*o, p*), paraldehyde (*q, w*), chloral hydrate (*r—t*), urethane (*u—v, x—z*). — × 500.

hours, but after 2 days normal cells preponderated. Traces of cf-growth were seen in 0,005—0,01 mol.

b. *New series* ($^2/_2$, 44). — Concentrations: 0,05, 0,10, 0,15, 0,20, 0,25, 0,30 volume % (0,006—0,04 mol). After 20 hours 0,20—0,30 % was dead, as were the majority of cells in 0,10—0,15 %. 0,05 % showed a lethality of 52 % and a rather high proportion of cf-forms. This concentration

began to ferment weakly after 2 days and reached full fermentation in 5 days. None of the stronger concentrations were then yet fermenting.

The cf-forms (Fig. 4 *o*, *p*) are initiated as tubes, but often develop into a more rounded form. The cells may acquire a rather normal shape but are larger than normally. The average type of cf-form is less striking than in ether and acetone. Chloroform is less efficient as cf-substance, since it acts toxically in those concentrations which give the reaction. This may also be the cause of the less extreme form of the cf-cells.

Tested zone: 0,0005—0,1 mol
Lethality: 0,02 —0,1 »
Toxicity: 0,006 —0,01 »
Cf-reaction: 0,005 —0,01 »

4. PARALDEHYDE.

a. *Orientating experiment* ($^8/_9$, 43). — Concentrations: 0,002—0,5 mol; lethality: 0,2—0,5 mol; cf-reaction: 0,05 (only solitary cf-forms) and 0,1.

b. *New series* ($^2/_2$, 45). — Concentrations: 0,5, 1,0, 1,5, 2,0 volume % (0,04—0,15 mol). None of these concentrations was lethal. 2 % was the only one which did not get into fermentation in 2 days. It was in weak fermentation, however, after 5 days. Cf-forms were found in 0,5—1,5 %, although rather infrequently.

The cf-forms are not very advanced (Fig. 4 *q*); often they consist only of one large cell and one outgrowing tube, often of the same breadth as the mother cell. A rather high frequency of giant cells with normal cell-form were found.

Tested zone: 0,002—0,5 mol
Lethality: 0,2 —0,5 »
Toxicity: 0,1 —0,15 »
Cf-reaction: (0,05)—0,1—0,15 mol.

5. CHLORAL HYDRATE.

a. *Orientating experiment* ($^8/_9$, 43). — Concentrations: 0,001—0,2 mol. Lethality: 0,2 mol. Also 0,1 showed no development in 2 days, but the cells were not dead. 0,02—0,05 mol had a high frequency of cf-forms after 20 hours, 0,01 had mostly normal cells although with clear cf-tendencies.

b. *Two complementary concentrations* ($^1/_2$, 44). — 0,04 and 0,06 mol. Both showed high frequency of cf-forms. The general development of the cultures was slow, 0,04 mol fermented after 3 days.

The cf-forms, easily produced in several concentrations of this substance, were often of a rather extreme type (Fig. 4 *r—t*). Long hyphae grow out and are retained as cells, from which new lateral hyphae are sent out. A certain regularity in the structure of the cf-colonies was thus acquired, as already discussed above in the general chapter.

Tested zone: 0,001—0,2 mol
Lethality: 0,2 »
Toxicity: 0,02 —0,1 »
Cf-reaction: (0,01)—0,02—0,1 mol.

6. URETHANE.

a. *Orientating experiment* ($^8/_9$, 43). — Concentrations: 0,01—2 mol. Lethality: 1—2 mol. 0,5 gave no development, some living cells remained after 2 days. In 0,2 mol there occurred plenty of cf-forms after 1 and 2 days, 0,1 mol had cf-forms only after 1 day, after 2 days the cells were again normal.

The cf-forms have an interesting type (Fig. 4 *u—v*, *x—z*). Very long hypha-tubes may, it is true, be found now and then, but these usually later on give rise to many small more or less rounded cells. After 2 days in 0,2 mol, for instance, numerous cf-colonies of 20 to 40 cells were found, all cells in the interior of the colonies being spherical (Fig. 4 *y*). They hang together even after heavy shaking and may be compared with the cf-colonies after long treatment with ethanol (Fig. 3: *18*). After some time the colonies tend to fall apart, and it is then seen that the size of the cells is very varying and that their outlines are often quite irregular.

Tested zone: 0,01—2 mol
Lethality: 1 —2 »
Toxicity: 0,2 —0,5 »
Cf-reaction: 0,1 —0,2 »

7. VERONAL, ADALINE AND SULFONAL.

These three substances — veronal (diethylbarbituric acid), adaline (*a*-bromo-*a*-ethyl-butyryl urea) and sulfonal (diethyl-sulfon-di-methyl-

urethane) — resemble each other in that saturated solutions do not give lethality and only little poison effect. Their cf-reaction is not very pronounced. In the strongest concentrations small groups of cells occur. The cells of these groups are often somewhat elongated in shape and are often larger than normally. In veronal I found a low percentage of developed cf-forms with distorted tube-like cells.

	Veronal	Adaline	Sulfonal	
Tested zone:	0,0005—0,08	0,00005—0,01	0,0001—0,02	mol
Cf-reaction:	0,08	0,01	0,02	»

In these concentrations the cf-reaction is only partial, the majority of the cells being normal.

8. A FEW OTHER SUBSTANCES.

Some data may be given for a few substances in which only preliminary and incomplete experiments have been made. Trichlorethylene and acethylene tetrachloride have been studied in the zone 0,003—0,03 mol. The latter substance gave cf-forms in 0,003 mol, which was the only concentration in the series permitting any development. Two halogen-substituted alcohols were studied, both used in practice as soporifics, viz. avertin (tribromoethyl alcohol) and chloretone (trichloro-*tert*-butylalcohol. Both gave typical cf-reaction in some concentrations.

	Avertin	Chloretone
Tested zone:	0,001—0,1	0,01—0,08
Lethality:	0,02 —0,1	0,03—0,08
Toxicity:	0,01	0,02
Cf-reaction:	0,002—0,005	0,01—0,02

9. SURVEY OF THE ALIPHATIC SUBSTANCES OTHER THAN THE ALCOHOLS.

All substances studied gave at least tendencies to cf-reaction in some concentration. The correlation between activity and water solubility, pointed out concerning the alcohols, is met with also here. The lethality, for instance, behaves in the following manner:

Substance	Solubility	Lowest lethal concentration
Acetone	∞	2,0
Urethane	11,25	1,0
Ether, chloral hydrate, paraldehyde ..	± 1	0,2—0,4
Chloroform	0,08	0,02

TABLE 2. *The cf-reaction of some aliphatic substances.*

Substance	Molar concentration	Number of cells per cf-colony												Total number of colonies	Average number of cells per cf-form	Percentage of cf-forms	Lethality percentage	Number of days to full fermentation
		1	2	3	4	5	6	7	8	9	10	12	15					
Ether	0,19	12	6	15	3	—	—	—	—	—	—	—	—	36	2,3	90,0	48,1	5
Acetone	0,41	15	9	6	1	2	1	—	—	—	—	—	—	34	2,1	89,7	47,3	5
	0,27	2	3	1	8	6	3	3	—	—	—	—	1	27	4,7	58,7	30,3	2
	0,14	1	1	2	2	—	2	—	1	—	—	—	—	9	4,1	2,7	7,5	2
Chloroform	0,0063	3	2	3	4	5	2	1	—	—	—	—	—	20	3,8	80,0	51,9	5
Paradelhyde	0,11	1	7	4	—	1	2	—	—	—	—	—	—	15	2,9	25,9	28,4	5
	0,08	1	2	2	2	1	7	4	3	1	1	1	—	25	6,0	8,0	7,9	2
	0,04	—	1	2	—	1	—	1	—	—	—	—	—	5	4,0	1,0	4,4	2
Chloral hydrate	0,02	—	1	5	6	1	—	2	—	—	—	—	—	15	4,0	51,7	23,7	2
	0,01	1	3	1	4	3	2	—	1	—	—	—	—	15	4,1	30,0	19,4	2
	0,005	1	3	3	1	—	—	—	1	—	—	—	—	9	3,1	15,3	15,7	2
	0,002	1	—	—	3	3	—	—	—	—	—	—	—	7	4,0	13,7	20,3	2
	0,001	—	—	4	3	1	—	—	—	—	—	—	—	8	3,5	17,8	21,1	2
Urethane	0,2	—	1	1	2	3								7	4,0	63,6	38,9	5
	0,1	not counted												—	—	57,7	4,6	2

The following concentrations of the various substances were in fermentation after 2 days and may thus be regarded as the upper limit of non-toxicity:

Acetone	0,27 mol
Urethane	0,10 »
Chloral hydrate	0,02 »
Ether	0,15 »
Paraldehyde	0,08 »
Chloroform	0,005 »

Broadly speaking, a clear negative correlation is valid also here between toxicity and water solubility. No close correlation can be expected in a group of such chemical heterogeneity. It is quite conceivable that, for instance, chloral hydrate shows a specific toxicity, falling outside the unspecific activity, which is correlated to the physical properties of the substances.

Table 2 gives a similar survey of the cf-reaction as was given in Table 1 for the alcohols. Those concentrations of the substances which have induced more than casual cf-forms have been recorded together with some data in connection with the reaction. In chloral hydrate the three lowest concentrations of Table 2 are taken from an experiment not recorded above. They refer to cultures grown at room temperature and are not quite comparable to the other values.

Substance	Active concentration	Maximal frequency of cf-forms	Average number of cells per cf-colony
Ether	0,19	90,0	2,3
Acetone	0,14—0,41	89,7	3,6
Chloroform	0,0063	80,0	3,8
Paraldehyd	0,04—0,11	25,9	4,3
Chloral hydrate	(0,001)—0,01—0,02	51,7	3,7
Urethane	0,1—0,2	63,6	4,0

The activity zones arranged according to water solubility:

Solubility	Activity zone
∞	0,14 —0,41
11	0,1 —0,2
$\pm$ 1	0,001—0,02; 0,04—0,11; 0,19
0,08	0,0063

A certain correlation between water solubility and activity is evident. Chloral hydrate also shows in the cf-reaction somewhat higher activity than its water solubility would indicate. As expected, the correlation is less distinct in this group than in the preceding group of substances.

V. ALICYCLIC SUBSTANCES.

1. CYCLOHEXANE AND BROMOCYCLOHEXANE.

Only one preliminary experiment was made with these two substances. Concentrations: 0,0001—0,005 mol. No concentration gave complete cf-reaction, but in 0,005 mol of cyclohexane and in 0,002 mol of bromocyclohexane colonies of rounded and irregularly shaped cells occurred.

2. CAMPHOR.

Camphor was the first substance in which the reaction was closely studied. It is still one of the most efficient cf-substances, especially when it comes to the induction of multicellular cf-colonies with long, tube-like cells. LEVAN and SANDWALL (1943) found activity in borneol also. Since it seemed of special interest to study substances related to camphor, several experiments were arranged in August, 1943, with various terpene derivatives.

Camphor was studied in the following concentrations: 0,000001—0,01 mol. A detailed testing was made of the zone 0,001—0,01. As already reported by LEVAN and SANDWALL (l. c.), almost complete lethality goes down to 0,008 mol. Below this concentration the cf-reaction reaches close to 100 %. A high degree of reaction is obtained down to 0,003 mol, below this concentration single cf-forms may be found as far down as 0,00001 mol. The lower threshold is thus rather indistinct. The frequency curve of cf-forms rises rapidly, however, at 0,002—0,003 mol (Fig. 5).

It is obvious that the cf-reaction is readily induced by camphor. It occurs in a large interval of concentrations and reaches high frequency in rather intoxic concentrations as well. The cf-cells may be very long tubes, and the colonies may reach a size which is visible macroscopically.

Tested zone:	0,000001—0,01	mol
Lethality:	0,008 —0,01	»
Toxicity:	0,005 —0,007	»
Cf-reaction:	0,002 —0,007	»

3. BORNEOL.

While camphor is a ketone, borneol is the corresponding alcohol. It is also efficient in giving the cf-reaction. Its lethal action goes somewhat lower down than that of camphor. Its zone of cf-activity is somewhat narrower. Cf-forms are found between 0,001 and 0,0045 mol, in high frequency only in the zone 0,0035—0,0045. It never reaches the completeness of the reaction induced by camphor.

Tested zone:	0,000001—0,01	mol
Lethality:	0,006 —0,01	»
Toxicity:	0,002 —0,0055	»
Cf-reaction:	0,001 —0,0045	»

4. BORNYL ACETATE AND BORNYL CHLORIDE.

These two esters of borneol are both inactive as far as the cf-reaction is concerned. They are decidedly more toxic than the preceding substances of this chapter.

	Acetate	Chloride
Tested zone:	0,0002—0,01	0,00001—0,001 mol
Lethality:	0,001 —0,01	0,0002 —0,001 »
Toxicity:	0,0006—0,0008	0,00008 »

5. CAMPHOR OXIME.

Here the keto-group ($>C=O$) of camphor is changed to an oxime ($>C=NOH$). It is lethal only in saturated solution plus a considerable precipitate corresponding to a concentration of 0,04 mol or higher. It has activity in a rather wide zone, but always in a low degree. The cf-forms are typical.

Tested zone:	0,00005—0,08	mol
Lethality:	0,04 —0,08	»
Toxicity:	0,003 —0,04	»
Cf-reaction:	0,003 —0,02	»

6. CAMPHORIC ACID.

This is a two-basic acid and it is the easiest soluble one of the camphor derivatives here tested. It gives lethality in 0,08 mol. In 0,02—0,04 mol numerous tendencies to cf-forms were met with after one day's treatment. There occurred elongated cells, single tube-like bud cells,

etc. Also after 2 days some cf-forms were seen in these concentrations, their frequency, however, being little.

Tested zone: 0,0001—0,08 mol
Lethality: 0,08 »
Toxicity: 0,01 —0,04 »
Cf-reaction: 0,02 —0,04 »

7. CAMPHENE.

This is an unsaturated hydrocarbon, and it is among the least soluble ones of these substances. It gives no cf-reaction.

Tested zone: 0,00004—0,004 mol
Toxicity and lethality: 0,0002 —0,004 »

8. TERPINEOL AND MENTHOL.

These substances, one saturated and one unsaturated alcohol, were tested. They gave no cf-reaction, terpineol may give some traces of reaction in 0,004 mol.

	Terpineol	Menthol
Tested zone:	0,000001—0,02	0,000001—0,01 mol
Lethality:	0,008 —0,02	0,002 —0,01 »
Toxicity:	0,002 —0,008	0,0006 —0,002 »

9. SURVEY OF THE ALICYCLIC SUBSTANCES.

Within the group of camphor derivatives an evident correlation is found between solubility and activity. It may be pointed out, as has already been done several times, that gross sources of error occur. The undeniable regularity of the solubility correlation indicates, however, that the fundamental principle underlying these conditions has not been obscured by the experimental uncertainty. Sources of error are, first, the difficulty in determining the water solubility of the substances. Unfortunately, for the substances concerned here the current tableworks very seldom give other values of solubilities than »very slightly soluble» or »insoluble». I had the possibility of making a rough estimate of the solubility when the series were mixed. The critical values — the weakest concentration with precipitate and the strongest one without precipitate — were controlled both at the preparation of the series and after keeping the solutions in shut bottles for one day. The more important substances were also controlled in solutions of distilled

water. Another source of error is the difficulty of obtaining pure substances. The camphor derivatives tested here were kindly presented by the Royal Technical College of Stockholm, where they had been produced during students' class work.

The camphor derivatives represent a rather wide scale of solubilities, and they are therefore well suited for a testing of the present working hypothesis. Arranged in decreasing solubility they will form the following series:

Substance	Solubility	Lowest lethal concentration	Highest non-toxic conc.
(1) Camphoric acid	0,02 —0,04	0,08	0,006
(2) Camphor oxime	0,008 —0,009	0,04	0,004
(3) Camphor	0,004 —0,005	0,008	0,003
(4) Borneol	0,001 —0,002	0,006	0,001
(5) Bornyl acetate	0,0008 —0,001	0,001	0,0004
(6) Camphene	0,0004 —0,0006	0,0002	0,0001
(7) Bornyl chloride	0,00008—0,0002	0,0002	0,00006

As seen, a striking correlation between toxicity and solubility is present.

Only four of the substances have cf-efficiency. It is rather suggestive that these four substances are those of the above series with the highest water solubility (Nos. 1—4). This indicates that the activity of the rest of the substances is cut off by the sinking solubility, as was found in the aliphatic alcohols: the activity line passes above the solubility line. The cf-reaction occurs in the following zones:

Camphoric acid	0,02 —0,04	mol (tendencies)
Camphor oxime	0,003—0,02	»
Camphor	0,002—0,007	»
Borneol	0,001—0,0045	»

As already mentioned, camphor and also camphor oxime may give single cf-forms in concentrations decidedly below those recorded above. Thus, in one experiment in 1942 camphor gave, counted as averages of $^1/_{25}$ mm^3, the following number of cf-forms in weak concentrations:

Concentration	0,00001	0,0001	0,0003	0,0007	0,001 mol
Number of cf-forms	0,8	0,8	1,2	3,6	0,8 »

The concentration 0,002 mol is considered as threshold value, since it is first in this concentration that any considerable frequency of cf-forms begins to appear.

TABLE 3. *The cf-reaction of camphor and related substances.*

Substance	Time of treatment	Molar concentration	Number of cells per cf-form: Aver-age	Number of cells per cf-form: Maxi-mal	Total number of cf-forms	Percentage of cf-forms	Lethality percentage	Number of cells in percentage of the control
Camphor	1 day	0,007	3,8	8	156	91,5	38,2	6,3
		0,0066	3,5	9	25	73,5	27,7	5,9
		0,006	3,5	9	159	85,0	32,2	4,9
		0,005	5,0	21	191	34,1	22,2	13,5
		0,004	3,9	12	113	5,1	2,3	33,2
		0,0033	3,2	7	22	6,5	6,6	45,7
		0,003	4,7	12	106	1,5	4,7	52,0
		0,002	4,8	13	51	0,7	2,5	61,3
		0,001	3,8	7	17	0,3	5,3	67,8
	2 days	0,007	4,4	30	432	18,5	4,9	22,0
		0,0066	4,9	20	62	6,1	7,9	17,1
		0,006	4,8	17	264	12,4	6,7	15,1
		0,005	4,2	19	186	4,5	3,4	31,5
		0,004	4,6	17	64	1,3	1,6	51,6
		0,003	3,5	18	91	0,6	2,7	74,7
		0,002	3,9	8	51	0,3	2,1	81,0
		0,001	2,7	5	41	0,1	3,2	116,3
Borneol	1 day	0,0045	2,6	6	11	33,3	78,2	3,7
		0,0040	3,1	9	140	22,5	40,3	4,6
		0,0035	3,9	12	130	21,2	17,5	5,4
		0,0030	3,8	12	183	6,6	5,6	24,2
		0,0025	4,3	8	66	1,9	4,8	30,8
		0,0020	4,0	10	27	0,5	4,4	39,9
		0,0010	2,0	5	8	0,5	0,7	60,3
	2days	0,004	5,5	11	10	3,3	50,0	1,1
		0,002	3,1	7	13	0,06	2,3	79,1
Camphor oxime	1 day	0,01	4,4	11	52	6,7	1,9	14,2
		0,009	4,4	16	62	4,5	1,8	25,5
		0,008	5,2	12	41	7,1	9,3	11,6
		0,007	4,6	10	49	4,7	4,6	19,8
		0,005	3,4	7	36	1,5	1,6	43,5
		0,004	2,7	5	35	1,1	1,2	58,7
		0,003	3,1	9	36	0,8	2,5	23,6

The cf-reaction of the four substances concerned shows some characteristic features. The cf-reaction induced by camphoric acid is very weak, only tendencies to cf-forms being noticed. Camphor oxime has a wide activity range, 0,003—0,02 mol. The reaction never becomes very predominating, however. In all concentrations normal cell-growth is most common, the frequency of cf-forms never exceeds 10 %. Camphor has a high frequency of cf-forms in a narrow zone, 0,006—0,007 mol. Here the cf-growth is almost 100 %. The frequency curve of

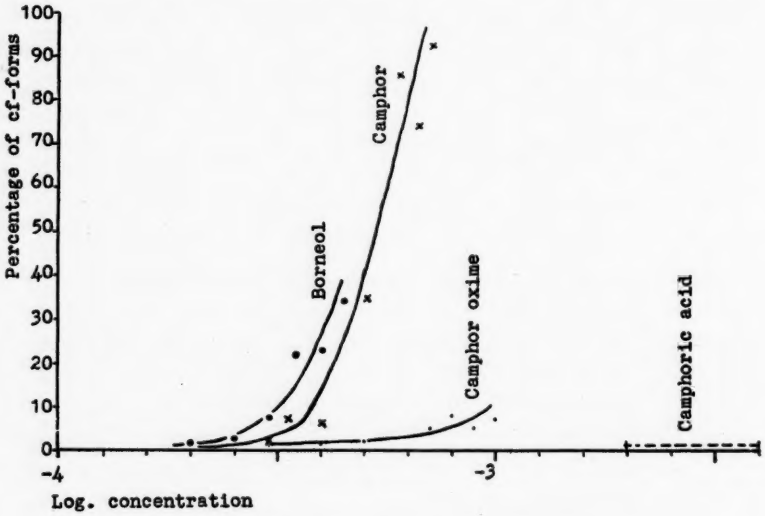

Fig. 5. Graph of the cf-activity of camphor derivatives.

cf-forms in borneol shows a similar steep increase as in camphor. It starts at 0,002 mol but is interrupted by lethality at 0,0045 mol. It has then reached 33 %. These conditions are represented graphically in Fig. 5.

Some results of cell counts in different concentrations of the alicyclic substances are collected in Table 3. Here are recorded the total frequency of cf-forms found in the cultures, the lethality and toxicity, the latter given in relation to the cell number of the controls. In camphor and borneol some data are given from some 2-day treatments as well. A summary of some of this material is already published in LEVAN and SANDWALL (l. c.).

The number of cells within the cf-forms is about the same in the three substances, 2—5 cells on an average. The variations are greater after 2 days, when the older cf-colonies have attained a higher cell number. At the same time the weaker concentrations no longer exercise any strong effect on the yeast, which evidently becomes resistant to the treatment, so here the cf-forms have a tendency to fall apart. This is most probably the cause of the low mean values during the second day in camphor and borneol. Even in the stronger concentrations of camphor there occurs after 2 days a considerably decreased lethality and increased cell number. Simultaneously, the frequency of cf-forms decreases owing to an acquired resistance towards the treatment.

It is clearly seen from Fig. 5 that among the substances tested here camphor represents optimum for the induction of the cf-reaction. It has a high frequency of cf-forms in such concentrations as show but little lethality.

VI. AROMATIC SUBSTANCES.

1. BENZENE.

Only explorative experiments have been made with benzene as with other aromatic substances tested. Most of the experiments with aromatic substances were made in August, 1943; some control series were made on later occasions too. Of benzene, concentrations from 0,004 to 0,016 mol were tested. The stronger solutions 0,013—0,016 were totally lethal, 0,004 mol had no perceptible effect, 0,008—0,01 mol had rather strong toxic effect. In the latter concentrations there occurred a varying frequency of small cell colonies of 4—8 cells without any striking deviation in cell-forms. This is the typical cf-reaction of aromatic monocyclic substances.

One benzene series laid down in July, 1946, contained the concentrations 0,007, 0,009, 0,010 and 0,012 mol. Especially 0,009 and 0,010 mol showed high frequency of cf-forms, while 0,012 mol showed little development in 1 day. Later on this highest concentration developed numerous cf-forms. Fig. 2 is drawn from cells treated 16 days in this concentration.

The cf-forms of benzene as of most aromatic substances do not have the elongated tube-like shape typical of the cf-forms induced by most aliphatic substances. The cells are rounded or have a more irregular form. The colonies never actually attain the size or cell number

of, for instance, camphor, which may depend on the active concentrations of the aromatic substances often being rather toxic.

Tested zone:	0,004—0,016	mol
Lethality:	0,013—0,016	»
Toxicity:	0,008—0,012	»
Cf-reaction:	0,008—0,012	»

2. BENZENE HOMOLOGUES.

In this series toluene, meta-xylene and mesitylene were studied. All of them gave cf-forms of the benzene-type. The cf-reaction of toluene was rather unclear.

	Toluene	Xylene	Mesitylene	
Tested zone:	0,002 —0,004	0,0008—0,0016	0,00018—0,00056	mol
Lethality:	0,0032—0,004	0,0013—0,0016	0,00046—0,00056	»
Toxicity:	0,0024—0,0028	0,0011	0,00035—0,00042	»
Cf-reaction:	0,0024	0,0010—0,0011	0,00035—0,00039	»

3. NITROBENZENE.

Only two concentrations were tested, 0,01 and 0,02 mol. The latter was lethal, the former had strong toxic effect and contained numerous typical cf-forms of the benzene-type.

4. ANILINE.

Six concentrations between 0,04 and 0,4 mol were tested. 0,08—0,4 mol had no development and probably had a lethal action. Only 0,04 mol showed some growth together with decided toxicity. This concentration gave almost 100 % cf-reaction.

5. PHENYL URETHANE.

This substance gave typical cf-forms, also sometimes with quite elongated cf-cells.

Tested zone:	0,002—0,02	mol
Lethality:	0,02	»
Toxicity:	0,002—0,016	»
Cf-reaction:	0,012—0,016	»

6. ACETOPHENONE.

This substance gave only partial cf-reaction in my experiments.

Tested zone:	0,005—0,05	mol
Lethality:	0,04 —0,05	»
Toxicity:	0,01 —0,03	»
Cf-reaction:	0,01	»

7. AROMATIC ALCOHOLS.

Two phenyl ethyl alcohols were tested, primary and secondary. The latter gave a clear cf-reaction, the former gave only tendencies.

	Primary	Secondary	
Tested zone:	0,001—0,2	0,001—0,2	mol
Lethality:	0,02 —0,2	0,05 —0,2	»
Toxicity:	0,01	0,02	»
Cf-reaction:	(0,005—0,01)	0,01—0,02	»

Thymol was also tested, and the related hydrocarbon cymene. Neither of them gave any cf-reaction.

	Thymol	Cymene	
Tested zone:	0,000001—0,01	0,000001—0,01	mol
Lethality:	0,002 —0,01	0,0005 —0,01	»
Toxicity:	0,0005 —0,001	0,0002	»

8. SURVEY OF THE AROMATIC SUBSTANCES.

Although the aromatic substances have given rather meagre results concerning the main subject of study, the cf-reaction, they have considerable interest for the general conclusions of the present work. They include substances with very varying water solubility and therefore give several good instances of the solubility-activity correlation.

It is of interest to study such a group of related substances as benzene and its methylated derivatives. These form a series of decreasing solubility from benzene with a solubility of 0,013—0,016 mol, determined in wort, to mesitylene, the solubility of which is some 40 times lower, 0,0004—0,0005 mol, determined and kindly communicated by ÖSTERGREN. The relation between water solubility, on one side, and lethality, toxicity and cf-reaction on the other, is evident:

Substance	Lowest lethal concentration	Highest non-toxic concentration	Cf-reaction
Benzene	0,013	0,004	0,008 —0,01 mol
Toluene	0,0032	0,002	0,0024 »
Xylene	0,0013	0,001	0,001 —0,0011 »
Mesitylene	0,00046	0,0002	0,00035—0,00039 »

If the eleven tested aromatic substances are divided into four groups according to their water solubility, the correlation is also evident:

Group	Substance	Solubility	Lowest lethal concentration	Highest non-toxic concentration	Cf-reaction
1	Aniline	0,1 —1,0	0,08	0,04	0,04
2	Phenyl ethyl alcohol Acetophenone Nitrobenzene Benzene Phenyl urethane	0,01 —0,1	0,01 —0,05	0,002 —0,005	0,005 —0,02
3	Thymol Toluene Xylene	0,001 —0,01	0,001 —0,01	0,0002—0,002	0,001 —0,0024
4	Mesitylene Cymene	0,0001—0,001	0,0004—0,0005	0,0001—0,0002	0,00035—0,00039

The agreement is actually greater than is seen from the above schematic table. If the substances are arranged in detail according to falling solubility, they are at the same time arranged according to falling lethality, falling toxicity and falling cf-threshold. In thymol the toxicity goes a few concentrations further down than expected from its water solubility. I do not wish, however, owing to the inaccuracy with which the water solubilities have been determined, to draw too far-reaching conclusions. The general tendency of the material is consistent, even if exceptions often occur in the details.

VII. MODIFICATIVE INFLUENCES ON THE CF-REACTION.

All treatments described in the preceding chapters have been made on yeast grown under optimal conditions. At the time of inoculation the yeast has been in vigorous fermentation, and immediately before it has been inoculated several times over on fresh wort. It has for a long period had good nutrition conditions. The experiments have been made at a constant temperature of 25 centigrades.

Now it was early noticed that even small changes in the environ-

ment could bring about considerable variations in the results of the experiments. Thus, some explorative experiments were made in Svalöf at room temperature (17—20°) without using a thermostat. It was found that cf-forms were obtained much more easily under these conditions than at 25°. Similarly it turned out to be hard to obtain cf-forms in ethanol if another yeast than »fuzzy heads» was employed. In the following pages a brief report will be given of three types of experiments which throw some light upon the significance of the general condition of the yeast, the influence of the temperature and, finally, the induction of a certain resistance of the yeast towards toxicity and cf-reaction.

1. THE PHYSIOLOGICAL CONDITION OF THE YEAST.

In these experiments three different types of bottom yeast were used, all belonging to the same clone as that of the above experiments:

A. »Fuzzy heads» (= hochkräus yeast),
B. Seed yeast from fermenting vessel,
C. Bottoms (cask deposit, »geläger» yeast).

A was taken from fermenting vessels during practical beer brewing at vigorous fermentation, B had sedimented and was taken immediately after finished fermentation, C had been stored for a couple of months in storage tanks.

The experiments were arranged in the following way: Series of test-tubes with 10 cc of wort containing different concentrations of the substances under experiment were simultaneously inoculated with approximately the same cell number of A, B and C. The course of the fermentation was followed daily. On various occasions samples were taken for microscopical control. Whereas the results concerning general toxicity were conclusive, I dare not yet deal with differences in cf-reaction.

a. *Methanol* ($^{27}/_{6}$, 45). — Concentrations: 5, 6, 7, 8, 9, 10, 11 volume % (1,2—2,7 mol). From earlier experiments it was known that 2 mol (about 8 %) does not come into full fermentation until after 17 days, i. e. a very strong toxic effect.

In A (fuzzy heads) no fermentation at all started during the first two days. On the 3rd day 5 and 6 % started, on the 4th day also 7 % and on the 6th day 8 % started, in the last case, a very weak fermentation. 9—11 % had no development at all. The only culture which had completed fermentation on the 6th day was 5 %.

The B series showed a striking difference: 5 and 6 % already started fermentation on the 1st day, and on the 2nd day all concentrations 5—11 % were in fermentation. The fermentation proceeded rapidly, so that on the 6th day all cultures had finished except 9—11 %, which were then in full fermentation. Yeast B (recently sedimented yeast) thus showed an immensely greater tolerance towards methanol than yeast A.

The C series (cask deposit) took an intermediary position. No fermentation occurred on the 1st day, but on the 2nd day 5—9 % started, and on the 6th day 5, 6 and 7 % had finished fermentation, 8—10 % were in full fermentation and 11 % had only just started.

b. *Ethanol* ($^{20}/_{6}$ and $^{27}/_{6}$, 45). — Two series were made both with the following concentrations: 5, 6, 7, 8, 9 and 10 volume % (0,9—1,7 mol). Earlier experiments had shown that 10 % did not arrive into fermentation in 17 days, while in this time 9 % showed a weak start of the fermentation.

The A series showed, as expected, similar behaviour as that just mentioned: No concentration started fermenting on the 1st day, 5—7 % started on the 2nd day, 8 % on the 4th, and on the 6th day even 9 % fermented although not so strongly as the four weaker concentrations. 10 % did not come into fermentation.

Within the B series fermentation started for 5—7 % already on the 1st day, and on the 2nd day all concentrations were in fermentation, the concentration 10 %, however, but hesitatingly.

The C cultures did not show fermentation until the 2nd day (5—8 %), on the 4th day 9 % started, 10 % did not get into fermentation. These differences are more clearly seen from the following table, which includes estimates of the cell numbers of methanol and ethanol. The estimates were made after one day's treatment:

%	A		B		C	
	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
5	0	1	10	10	2	4
6	0	1	6	8	2	3
7	0	1	3	8	2	1
8	0	0	2	7	1	1
9	0	0	2	2	2	0
10	0	0	3	3	1	0
11	0	—	3	—	0	—
0 (control)	10	10	10	10	10	10

c. *Tertiary butanol* ($^{27}/_{6}$, 45). — Concentrations: 0,5, 1,0, 1,5, 2,0, 2,5, 3,0 volume % (0,65—0,32 mol). It was known that 0,2 mol (1,8 %) ferments after 2 days, while 0,5 mol (4,5 %) shows no development in 6 days.

All series were in fermentation on the 2nd day. The onset of the fermentation was different. In the A series fermentation started only in 0,5 % on the 1st day, in the B series all started, and in the C series 0,5 and 1 % started on the 1st day. The number of yeast cells was estimated after 24 hours with the following result:

Concentration	0	0,5	1,0	1,5	2,0	2,5	3,0
A	10	6	2	2	1	1	0
B	10	10	10	10	10	8	3
C	10	10	7	2	1	1	1

d. *Potassium cyanide* ($^{29}/_{6}$, 45). — In order to illuminate the question whether the differences in reaction of the yeast under various conditions are caused by a more specific resistance to such substances as may occur normally in the metabolism of yeast, or by a more general power of resistance to environmental disturbances which may affect the yeast cell so to say more unexpectedly, an experiment similar to the preceding ones was performed with potassium cyanide.

The reaction of yeast to this substance was tested in advance in a series of 13 concentrations of potassium cyanide, ranging from 0,0001 to 1 mol (0,00065 to 6,5 weight %). The experiment was performed in FREUDENREICH flasks with 10 cc of wort in each. No fermentation occurred after one day in any concentrations. After 2 days 0,0001—0,001 were in full fermentation, 0,002 mol contained living cells, while 0,005 mol and higher concentrations seemed to be killed. Since potassium cyanide may induce both c-mitosis and c-tumours in *Allium* (LEVAN, unpublished), it was of special interest to look for cf-forms in these treatments. In 0,002 mol certain tendencies to simple cf-forms were seen, but hardly in such a frequency that I would care to draw any conclusions.

The experiment with different yeast types was arranged with the following concentrations of potassium cyanide: 0,001, 0,002, 0,003, 0,005, 0,007, 0,01 mol, thus, a scale reaching considerably above the lethality limit of the preceding experiment, in which a yeast from the fuzzy heads stage had been employed.

The differences found between the different yeast types were striking. The A series agreed with the above explorative series: No fermentation on the 1st day, fermentation in 0,001 and 0,002 on the 2nd

day, 0,003 on the 3rd day. On the 4th day 0,003 fermented vividly, while fermentation had finished in 0,001 and 0,002, which were already completely fermented. The microscopic control showed that in the three higher concentrations which did not show any fermentation 0,005 had a beginning development, 0,007 contained living cells, while 0,01 mol seemed killed.

The B series behaved quite differently: On the 1st day fermentation already started in the 4 weakest concentrations, 0,001—0,005 mol, and on the 2nd day all concentrations were well started. On the 4th day the 4 weakest concentrations were finished, while the 2 strongest were in full fermentation. A definitively greater tolerance towards the toxic effect of potassium cyanide was thus demonstrated in the B yeast than in the A yeast.

The C series resembled the A series. In C 0,005 mol also came into full fermentation on the 4th day, while 0,007 and 0,01 never started fermentation and nor did they show any cellular development. Although giant cells were not infrequently met with in these treatments, real cf-cells were very seldom seen.

Summarizing the results of these experiments, it may be stated that in the determination of different threshold concentrations it is of great importance to employ a yeast type which is not only genetically uniform but which is also, as far as possible, in the same physiological condition. The same genotype reacts highly differently if it is in brisk division and full fermentation, if it is at rest after completed fermentation, or, finally, if it has been stored for a long time under conditions favouring autolysis. The few orientating experiments recorded above all give the concordant result that yeast at the stage of the fuzzy heads is most sensitive to noxious effects, while the yeast is most resistant immediately after finished fermentation. The difference in reaction shown between those two types of yeast may represent a change in toxicity threshold from 1 : 2 or 1 : 3. The »geläger» yeast takes an intermediate position in all experiments, the deficient nutrition having evidently exhausted the resistance which it had soon after the end of the fermentation.

2. THE INFLUENCE OF THE TEMPERATURE.

As already mentioned in the introduction, it has for long been known that beer yeast has a tendency to grow out to more elongated cells under cold conditions and to form more rounded cells in warmth. This elongated growth in cold may sometimes pass over into an un-

doubted tendency to cf-growth. The detailed conditions under which yeast may give cf-reaction induced by cold are, however, not known.

In order to study the influence of the temperature factor on the cf-reaction I arranged some experiments in February, 1944. They gave the decided result that cold has a general tendency to decrease the threshold value of the cf-reaction induced by various chemicals, while warmth has a tendency to heighten it. These experiments were made in the same manner as already described above. 100 cc of wort with various concentrations of the substances were inoculated with yeast from cultures in vivid fermentation. Three series of each substance were cultured, each of them being kept in a thermostat under the following temperatures respectively:

E: 9°, F: 18—20°, G: 30°.

Since the cf-reaction depends on the developmental stage of the culture — it is easiest to study in the beginning of the growth — the comparison between the three series could not be made after the lapse of the same time. F and G were examined on the 1st day after the inoculation, E on the 2nd to 4th day.

a. *Ethanol* ($^4/_2$, 44). — Concentrations: 1, 2, 4, 6, 8 volume % (0,2—1,3 mol). The lethality is here as in all the other temperature experiments especially high in the highest concentrations of the G series. It is clear that the same concentration of a narcotic substance acts more destructively at 30° than at lower temperatures. The average number of cf-forms in $^1/_{25}$ mm^3 was counted in the three series. It is given together with the percentage of cf-forms calculated on the number of living cells (values within brackets):

Concentration:	1	2	4	6	8	Average
E	12,4 (74)	10,8 (77)	6,0 (83)	7,6 (86)	0,4 (17)	7,4 (67)
F	6,0 (30)	8,4 (70)	10,8 (96)	7,6 (79)	1,6 (27)	6,9 (60)
G	0,3 (0,5)	3,2 (1,6)	3,2 (44)	0,8 (6)	0	1,6 (10)

The frequency of cf-forms is evidently lower in the G series. To this comes the fact that both the cell-shape in general and that within the cf-forms were shorter and more rounded in this series. The cf-forms could often not be identified from their cell-form but only from their property of not falling apart when shaken.

b. *Tertiary butanol* ($^5/_2$, 44). — Concentrations: 0,2, 0,6, 1,0, 1,4, 1,8 volume % (0,02—0,2 mol). The following number of cf-forms was recorded:

Concentration:	0,2	0,6	1,0	1,4	1,8	Average
E	9,6 (55)	8,4 (64)	9,6 (96)	12,0 (97)	12,0 (100)	10,3 (82)
F	10,8 (35)	8,8 (43)	14,4 (82)	12,6 (97)	20,4 (98)	13,4 (71)
G	0	6,8 (4)	11,6 (17)	11,2 (52)	6,4 (53)	7,2 (25)

When the percentage of cf-forms on the total number of living cells is calculated, it appears that the curves of the three types of treatment run rather parallelly: E uppermost, G lowest. They all have their main increase in the same concentration region, 0,6—1,4 %.

In this experiment the difference in appearance of the cf-forms

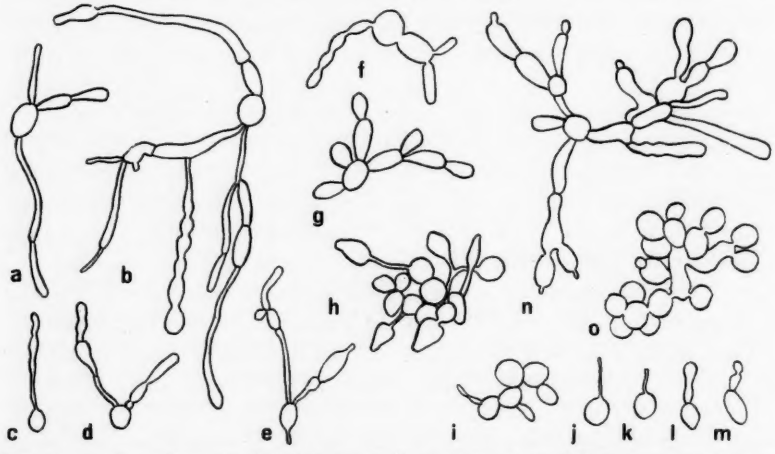

Fig. 6. *a—h.* cf-growth induced by tertiary butanol at different temperatures; *a*: 0,6 % for 3 days at 9, *b*: 1 % for 2 days at 20, *c—e*: 1,4 % for 20 hours at 20°, *f—h*: 1,4 % for 20 hours at 30°; *i—m*: ethyl ether 1 % for 2 days at 30°; *n*: cf-colony from yeast grown on ordinary wort and moved into 1,5 % tertiary butanol, *o*: cf-colony from yeast pre-treated for 15 days with tertiary butanol, thereafter moved over to 1,5 % tertiary butanol. The cell-shape of this cf-form is not elongated. — × 500.

originating from the different series was even more striking than in the ethanol series. In E and F the cf-forms had the appearance earlier described with long narrow, tube-like cells (Fig. 6 *a—e*); in G, on the other hand, a more rounded cell-shape predominated (Fig. 6 *f—h*). Often the cell-size of the rounded cells as well is larger than normally. One such rounded cell may send out radiary tubes, all of which soon swell up forming new rounded cells. These colonies may assume fantastic forms with alternating narrow tubes and swollen, spherical or pear-shaped cells (Fig. 6 *h*).

c. *Ether.* ($^7/_2$, 44). — Concentrations: 0,0001—0,05 mol. The lethality was considerable in all three series, only in 0,05 mol at 30° was it almost total. The average lethality of the three series was:

E: 28,9 %, F: 14,7 %, G: 26,0 %.

The increase in cell number was about the same in most cultures; only the weak concentrations of the warmth series showed a much stronger cell proliferation.

In E and F cf-forms were found all through the series, in G they were much rarer and of a less extreme type (Fig. 6 *i—m*). The following numbers were counted in $^1/_{25}$ mm^3:

Concentration:	0,0001	0,0002	0,0005	0,01	0,02	0,05	Mean
E	6,8 (42)	6,4 (48)	5,2 (68)	4,8 (80)	5,6 (70)	2,4 (50)	5,2 (59)
F	3,2 (18)	2,8 (14)	3,6 (15)	6,0 (30)	6,0 (52)	0,8 (18)	2,7 (25)
G	0,4 (0,2)	0 (0)	1,6 (0,1)	0,8 (1)	0 (0)	0,8 (20)	0,6 (3,6)

d. *Urethane* ($^9/_2$, 44). — Concentrations: 0,01—0,5 mol. The lethality was high (97 %) only in the highest concentration of the G series. On the other hand, the cell number counted after 20 hours was especially high in the three lowest concentrations of F and G. In E the cell number had about the same order of magnitude in all concentrations when counted on the 2nd day.

The cf-forms were commonest in E, where all concentrations except the lowest one had them. In F they occurred in 0,1 and 0,2 mol in rather high frequency, in G only in 0,2 mol. The threshold may be said to vary with the temperature in the following manner:

9°	0,02 mol
18—20°	0,1 »
30°	0,2 »

The round cell-form of the cf-forms of urethane may have made the recognizing of all cf-forms difficult. In the F series, too, the shape of the cells was mostly quite round. The following numbers of cf-forms were found (percentages in brackets):

Concentration	0,01	0,02	0,05	0,1	0,2	0,5
E	0	2,8 (20)	3,6 (15)	7,2 (75)	0,4 (14)	—
F	0	0	0	24,0 (58)	2,8 (64)	0
G	0	0	0	0	3,2 (73)	0

All these experiments on the cf-reaction under influence of different temperatures, however preliminary, show the same general tendency. The induction of the cf-reaction is facilitated by low temperature and is impeded by high temperature. Since in some cases low temperature alone has evident tendencies to favour cf-growth, it may be concluded that cold adds to the effect exerted by the various chemicals. Therefore a lower concentration is sufficient under cold conditions to get the same effect as, at a higher temperature, would have been induced by a higher concentration.

3. INDUCED RESISTANCE TO THE CF-REACTION.

One feature common to all treatments of yeast with narcotic substances is that the threshold of toxicity as well as of the cf-reaction does not remain constant during the course of the experiment but has a tendency gradually to rise. Such concentrations of a substance as during the 1st day give only weak development in cell number and high frequency of cf-forms often give during the 2nd day full development in cell number and now preponderantly normal cells and very few cf-forms. Evidently something happens which brings about either a decreased concentration of the substance in the culture medium or a greater tolerance (= increased threshold) of the yeast cells towards the treatment.

Since this peculiarity of an upward shift of the thresholds during the experiments is characteristic of all the different types of treatment, it is *a priori* hardly probable that the yeast cells have the ability through their own metabolism to remove or otherwise make harmless such a proportion of the various substances that the concentration which they have in the surrounding medium will fall below the threshold value. The other alternative, which involves an acquired resistance of the yeast cells, seems more plausible, and is in agreement with the conditions of many other types of narcotic and toxic reactions. Several experiments have been arranged to test whether the culturing of yeast in a medium containing a certain substance makes the cells more resistant to this substance, and perhaps also to other substances. Broadly speaking, all experiments indicate that such is the case. However, many disturbing factors are at work in these types of experiments, so I cannot as yet consider the problem as solved. I hope to continue the work along this line, and for the present I will only mention in all brevity one preliminary experiment made in February, 1944. This may indicate one line of approach to this important problem.

A number of yeast cultures were given various pre-treatments. They were grown for a fortnight on wort containing such concentrations of various substances as initially brought about a strong checking of the propagation of the yeast and also gave a high frequency of cf-forms but as later on permitted a good development of the yeast and full fermentation. These solutions were the following:

1. Ethanol ..... 1,2 mol
2. Tertiary butanol.. 0,2 »
3. Lauryl alcohol .. 0,01 » (saturated, with excess of substance)
4. Acetone ..... 0,41 »
5. Ether ..... 0,19 »
6. Chloroform ..... 0,0063 »
7. Paraldehyde .... 0,15 »
8. Pure wort as control

Yeast from these cultures was now inoculated on wort with three of the above substances, viz. ethanol, acetone and ether, in the above concentrations. The following estimate of the fermentation and the frequency of cf-forms was made on the 3rd day:

Pre-treatment	Ethanol		Acetone		Ether	
	Fermentation	Cf-forms	Fermentation	Cf-forms	Fermentation	Cf-forms
Ethanol	+	1	±	7	—	no development
Butanol	+	0	—	0	—	» »
Lauryl alcohol ..	±	0	+	0	—	» »
Acetone	+	1	++	0	±	2
Ether	—	0	±	1	—	no development
Chloroform	+	2	—	0	—	» »
Paraldehyde	+	1	±	2	—	» »
Pure wort	+	5	+	10	—	» »

Some interesting facts are seen from this. The acetone-yeast gives the strongest fermentation in the acetone-series, and is the only one which shows any development at all in the ether series. Although the control yeast gave a high frequency of cf-forms in acetone, the acetone-yeast had no cf-forms. The development of the cf-forms was throughout more extreme within the control yeast than in any of the pre-treated yeasts. As an instance of this Fig. 6 *n* shows a typical cf-form from the control yeast in 1,5 % tertiary butanol, while Fig. 6 *o* shows a cf-form of the butanol-yeast under the same treatment. The former cf-

colony has the shape typical of such a treatment, while the latter, although it has formed a cf-colony, consists of normal cells.

These results point to an evident unspecificity of the induced resistance. All kinds of pre-treatments have resulted in some degree of resistance to ethanol and acetone, the untreated yeast giving the most extreme cf-growth in both series. It must be stated that the experiments on pre-treatments of yeast with different substances point in the same direction as tests of the yeast in different physiological conditions. As in these experiments, here too, a certain unspecific resistance may be obtained by the yeast.

VIII. CONCLUSIONS.

The present morphological and experimental work on the cf-reaction of yeast leads to the following points and conclusions:

(1) The very type of the reaction suggests a disturbance or an irregularity of the growth, a switching-off of the growth control, which under normal conditions is responsible for a regulated cellular growth and propagation.

(2) This switching-off of the growth control may be more or less complete: in some cases the entire growth results in long irregular threads or swollen sacs, in other cases a certain growth pattern is maintained, recalling the morphology of other *Saccharomyces* species. By this fractionated withdrawal of different elements of the growth control a certain analysis of the normal growth may be attained.

(3) The cf-reaction is reversible: if the concentration of the active substance in the culture medium sinks below the threshold value of the reaction, or if the yeast cells acquire resistance to the treatment, the same cells as were earlier under command of the reaction, start normally to bud off daughter cells, which in the sequence behave normally.

(4) The nuclear divisions of the cf-forms are to a certain degree independent of the cellular disturbances. Normal mitoses may be found during a prolonged cf-growth. However, nuclear disturbances also gradually appear in strong concentrations of various substances. The commonest types of these disturbances are the following: a considerable increase in chromosome number of the nuclei, irregularities in the mitotic mechanism, multipolar spindles, vagabonding chromosomes, origin of micronuclei, etc. Whether regular c-mitoses occur is still a matter of doubt. The presence of tetraploid nuclei in otherwise normal cells, which was observed after rather short treatments, makes it per-

ceptible that the threshold of c-mitosis in these organisms lies close to the lethality limit. It is possible that in strong treatment single c-mitoses are immediately induced, thereupon followed by an acquired resistance, which moves the c-mitotic threshold above the lethality limit. The nuclear disturbances appearing later on have the same general type as weak c-mitotic disturbances in which the spindle is made somewhat ineffective but is not totally narcotized. The high chromosome number often seen in the nuclei of old cf-cells, as well as the long resting stage which these nuclei are seen to go through, indicate endomitotic processes. In the same manner as is known for higher plants and animals the enormous increase in cellular volume may bring about an adjustment of the chromosome number of these cells.

It may be stated that parallel with the disturbed control of the cellular growth, though not necessarily dependent thereon, disturbances also occur in the mitotic control, disturbances of the same type, but less complete than the c-mitosis of higher plants.

Certain types of induced mitotic disturbances made it possible to observe the size of single chromosomes. This condition gave a key to the normal chromosome number of the yeast studied, which was estimated to at least ten, possibly higher.

(5) The mitotic disturbances are also reversible. If an increase in chromosome number has taken place, the higher number is of course maintained, but after the end of the treatment the normal course of mitosis is restored. It is true that in SANDWALL's and my experiments no permanent polyploid yeast strains have been isolated, which is probably due to the fact that relatively little work has been done by us in that direction. Yeast types with in all probability polyploid chromosome number have originated on several occasions in the work of other authors.

(6) The cf-reaction is not confined only to a few certain chemicals, camphor and some derivatives of camphor. In the present experiments it has been induced by some fifty substances, aliphatic, alicyclic and aromatic. Most of the substances are of the type known for their narcotic properties.

This fact alone indicates that the reaction is not tied to any certain chemical constitution. It rather suggests that certain physical properties common to the efficient substances may be of significance.

(7) This conclusion is strengthened by the persistently observed correlation between physical properties (water solubility) and threshold value of the reaction. The reaction is generally induced by concen-

trations which lie in the vicinity of the saturation point of the substance, only seldom does it go below 0,1 of this concentration. According to the view of FERGUSON (1939), this corresponds to a thermodynamic activity of 1—0,1, which is characteristic of unspecific, physical action.

Although this condition may be the rule, it does of course not exclude the fact that some substances give effect at decidedly greater distances from the point of saturation. The lower aliphatic alcohols, acetone, chloral hydrate, chloroform and many others may have thermodynamic activities indicative of a more specific, chemical action.

It is possible that the common features of the reaction are caused by a physical action common to all the acting substances. The manifold deviations in type of the reaction are perhaps induced by specific chemical groups in the substances.

(8) A certain unspecific resistance to the reaction may be induced. Cells which have gradually been accustomed to an increasing content of ethanol (yeast from the sediment immediately after completed fermentation) are found to be more resistant not only to ethanol and butanol, but also to potassium cyanide, than cells which have for a long period grown only on wort without any considerable content of ethanol.

(9) Cold, alone or in combination with chemical treatments, imparts to the cells increased tendencies to give the cf-reaction.

Through these studies the cf-reaction, which has hitherto occupied rather an isolated position, has been brought in line with the also normally occurring phenomena of involution growth of yeast and of the morphological deviations in cell type induced by cold and other environmental conditions. Furthermore, these phenomena are now comparable to certain other reactions of living organisms, caused by various external factors. For instance, the cf-growth of the cells is of similar kind to the c-tumour reaction of higher plants, the nuclear disturbances of the cf-reaction are comparable, if not identical, with the c-mitotic reaction.

The cf-reaction may consequently be regarded as a partial reaction of the important complex known as the narcotic reactions. Common to all of these is the reversible switching-off of higher, controlling functions, the unspecificity of the induction, the correlation with physical properties of the inducing substances, the possibility of acquiring resistance. In addition to the general narcotic reactions of animals and

the more specific reactions just mentioned of c-tumour, c-mitosis, cf-growth, there also belong here many forms of general toxic action. Common features are easily found between the narcotic reactions and several bactericidal and insecticidal reactions, as has often been pointed out in the literature. If these narcotic-toxic reactions are allowed to extend their action so far that vital functions are held up, which cannot be switched off without serious damage, death by narcosis will be the result.

The individual character of each of the more specific reactions as, for instance, the cf-growth is given through the interrelation of their activity thresholds with toxicity and lethality. The greater the distance is between the threshold of the specific action, on one hand, and toxicity and lethality, on the other, the easier is the reaction obtained, the more extreme is its expression, the more efficient is the inducing substance said to be.

Some persistent features pointed out in connection with certain groups of related substances treated above, either homologous series, as the aliphatic alcohols, or derivatives of a certain substance, as the camphor group, may receive some illumination from these conditions. It has been mentioned that in such groups of substances an optimum may exist for the induction of the reaction. Thus, among the alcohols, butanol and pentanol represented the optimum, in the camphor group, camphor. Towards both sides substances occurred which gave the reaction less completely and with greater difficulty. The nature of these optima may be understood when viewed in relation to the different reaction limits just dwelt upon. At the optimum the maximal distance must be present between the activity threshold and the toxicity threshold.

On moving within the series towards the lower (= more water soluble) members (in our instance towards methanol or camphoric acid), the activity threshold will move upwards, higher concentrations will be needed to attain the effect. The unspecific poison effect also shifts upwards, but here there appears a specific toxicity, known, for instance, for the lower alcohols from other biological reactions. The curve of the toxicity threshold will consequently bend downwards and converge towards the activity threshold. This may be the cause of the diminished activity in the lower members of a series.

On moving in the opposite direction from the optimum, that is towards the higher (= less water soluble) members of the series, the activity threshold, as well as the toxicity threshold, sinks, though less

rapidly than does the solubility. Consequently, a point is reached at which the activity line crosses, and passes above, the solubility line, and the same will happen with the toxicity curve. To the left of this point the solubility will be too low to give the reaction, or, as the case may be, to give poison effect, the well-known cut-off of activity (cf. FERGUSON, l. c.), of toxicity or of lethality is reached. In our instance the cf-reaction is cut off between octanol and cetyl alcohol, and between borneol and bornyl acetate. Already at octanol it was noticed that the toxicity threshold, the cf-threshold and the solubility threshold were crowded together, resulting in a very narrow amplitude of the cf-reaction. In cetyl alcohol neither the cf-reaction nor any high degree of toxicity was obtainable. In the camphor group, on the other hand, even the least soluble substances had lethal effect in some concentration. Here the toxicity line had not yet crossed the solubility line, this point of crossing in the case of camphor derivatives evidently lying far to the left from the point of the cut-off of the cf-reaction.

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CONTENTS.

Page

I. Introduction 457

II. The morphology of the camphor reaction 460
1. Cellular morphology 460
2. Nuclear morphology 463

III. The aliphatic alcohols 471
1. Methanol 471
2. Ethanol 472
3. Propanol 474
4. Butanol, C_4H_9OH 474
5. Pentanol, $C_5H_{11}OH$ 476
6. Octanol, $C_8H_{17}OH$ 477
7. Lauryl alcohol (dodecanol, $C_{12}H_{25}OH$) 477
8. Higher alcohols 478
9. Survey of the aliphatic alcohols 478

IV. Other aliphatic substances 481
1. Ethyl ether 481
2. Acetone 482
3. Chloroform 483
4. Paraldehyde 484
5. Chloral hydrate 484

Page

6. Urethane 485
7. Veronal, adaline and sulfonal 485
8. A few other substances 486
9. Survey of the aliphatic substances other than the alcohols 486

V. Alicyclic substances 489
1. Cyclohexane and bromocyclohexane 489
2. Camphor 489
3. Borneol 490
4. Bornyl acetate and bornyl chloride 490
5. Camphor oxime 490
6. Camphoric acid 490
7. Camphene 491
8. Terpineol and menthol 491
9. Survey of the alicyclic substances 491

VI. Aromatic substances 495
1. Benzene 495
2. Benzene homologues 496
3. Nitrobenzene 496
4. Aniline 496
5. Phenyl urethane 496
6. Acetophenone 497
7. Aromatic alcohols 497
8. Survey of the aromatic substances 497

VII. Modificative influences on the cf-reaction 498
1. The physiological condition of the yeast 499
2. The influence of the temperature 502
3. Induced resistance to the cf-reaction 506

VIII. Conclusions 508
Literature cited 512

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THE CYTOLOGICAL EFFECT OF CHLOROFORM AND COLCHICINE ON ALLIUM

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I. INTRODUCTION.

BY introducing the concept of thermodynamic activity into toxicology FERGUSON (1939) arrived at a division of the various poisons into two classes, those with preponderantly physical and those with preponderantly chemical action. Since thermodynamic activity of a substance in solution may be roughly expressed as the relation between the lowest active concentration and the solubility, it is important to know the solubility of substances which are employed as agents.

The information given in the literature concerning the water solubility of colchicine is rather varying. According to the fifth Swiss Pharmacopoeia, one part of colchicine may be dissolved in 70 parts of water. The Handbook of Chemistry and Physics (1944) gives the water solubility as 4,5 %, MÜLLER (1903; see LEVAN and ÖSTERGREN, 1943) gives it as 9,6 %, ZEISEL (1886), finally, states that colchicine may be dissolved in water in all proportions, these being only a few instances of references in the literature to the solubility of colchicine. These apparent contradictions receive their explanation in the property of colchicine to form crystals which contain various solvents. As early a worker as ZEISEL (l. c.) found that colchicine obtained from a chloroform solution will form crystals with a certain content of chloroform. Such a type of colchicine will show a decreased water solubility. By heating with water the compound is again split into its components. On evaporating the water solution the chloroform disappears and a chloroform-free colchicine is obtained. If a strong colchicine solution is left for some time, the alkaloid precipitates with crystals containing $^1/_2$ mol of crystal water. This form is the one referred to in the Swiss Pharmacopoeia, and the solubility 1 : 70 is valid for this form. Over sulfuric acid in a vacuum the crystals gradually lose their water and change into the form free from solvent, which form is of unlimited water solubility. The solubility of this amorphous colchicine decreases, according to ZEISEL, with increasing temperature: thus at 82° it is only

12 %. The following forms of colchicine may be recorded together with their water solubilities:

Pure (amorphous) colchicine	unlimited
Colchicine + $^1/_2$ mol H_2O	1 : 70
Colchicine + $^1/_2$ mol $CHCl_3$	about 8 %
Colchicine + 1 mol $CHCl_3$	about 4 %

The content of solvent in colchicine is of no great consequence for the practical polyploidizing experiments, although it is probable that the chloroform-containing form is more poisonous than the pure colchicine, and therefore may be somewhat less suitable for some purposes. On the other hand, these conditions must be of great importance when quantitative investigations on the effect of colchicine are concerned. Thus, colchicine with 1 mol chloroform contains 25 % by weight of chloroform. Only in exceptional cases have the workers in this field given information as to what kind of colchicine has been employed, and only rarely has a direct comparison been made between the different forms of colchicine. BRUES and COHEN (1936), in their study of the active group of the colchicine molecule, test chloroform-containing and chloroform-free colchicine on regenerating rat liver. They obtained from the two treatments »entirely similar results as far as the cytological picture was concerned». BOAS and GISTL (1939) point out that in higher concentrations of crystalline colchicine the chloroform content cannot be disregarded. MANGENOT (1942) compared crystalline and amorphous colchicine in concentrations of 0,05 and 0,025 % without observing any differences in the effect on *Allium* roots. MAIROLD (1943) found no difference in the influence on plasmolysis between the two forms of colchicine. On the other hand, a distinct difference was shown in the effect on the growth of *Lupinus* roots of very low concentrations. While 0,001 % of amorphous colchicine acted stimulating on the root growth, the same concentration of crystalline colchicine gave only growth retardation. At higher concentrations as, for instance, 0,005 % both forms gave an equal retardation. MAIROLD assumes that in this case only very weak concentrations allow the effect of chloroform to come to light, in higher concentrations the effect of colchicine will predominate. MAIROLD cites a work of JOSING (1901) which showed that also in very low concentrations (as low as 0,0001 %) chloroform may have an effect on the plasmatic current.

In the course of our studies of c-mitotic effects on various liliaceous plants we have directed some attention to differences in action of

amorphous colchicine and crystalline colchicine. This point has been tested both in plants sensitive to colchicine and such as are known for their resistance to this substance. In the present paper only the first case will be considered, the effect of chloroform alone, chloroform-containing colchicine and chloroform-free colchicine being studied. As experimental object root-tips of *Allium Cepa* have been employed.

II. THE EFFECT OF CHLOROFORM.

The chloroform employed was immediately before use deacidized with sodium carbonate, thereafter washed with distilled water until neutral reaction was given, dried over $CaCl_2$ and fractionated. A series of eight concentrations from saturated solution (1 % by weight) down to 0,005 % was studied. As regards the cytological effect, the two strongest solutions 1 and 0,5 % were of considerable interest and must therefore be described in some detail. In these concentrations the roots rapidly became soft. Cytologically the picture suggests a fixation effect, the various mitotic stages were kept as they were at the moment the root-tips were killed. No c-mitotic effects occurred, nor any reversions of the contraction stages into despiralized stages.

The appearance of the resting nuclei was striking (Fig. 1 *a*, *b*). All through the meristem the resting nuclei were provided with irregular outgrowths into the plasm, resembling the precipitation membranes of »chemical gardens». These protuberances, as also the entire nuclei, were often quite devoid of any chromosomal structures. Just the centromeric heterochromatin was, however, often left (Fig. 1 *a*, *b*). In some nuclei chromosome threads occurred, these often being extended through the nuclear protuberances into the plasm (Fig. 1 *c*).

The contraction stages of the chromosomes, metaphase—anaphase, are characterized by a gradually proceeding dissolution of the chromatic material. In some pictures (Fig. 1 *d*) it was clearly seen that the matrix material left the chromosomes and wandered out into the plasm. The chromonema, or genic thread, apparently showed a greater resistance to the dissolution. Often these parts of the chromosomes were extremely delicately stained, giving an unusually clear picture of the internal structure of the chromosomes. Although the structures thus revealed must be considered as lethal, they are according to our opinion of a certain significance for the understanding of the vital structures. Fig. 1 *e* shows one cell at early metaphase, where most of the matrix is melted away, *f*—*j* show other instances of metaphase chromosomes,

k—*n* are anaphase chromosomes. A feature common to all these pictures is the subdivision of the chromosomes into chromatids, half-

Fig. 1. *a*—*c*: resting nuclei treated with 0,5 % chloroform for 4 hours; *d*: metaphase, same treatment; *e*—*j*: metaphase, *k*—*n*: anaphase chromosomes, ½ hour in 1 % chloroform; *o*—*q*: metaphase; *r*—*s*: anaphase, 4 hours in 0,5 % chloroform; *t*: bow metaphase, 4 hours in 0,25 %; *u*: c-metaphase with strong chromosome contraction, 24 hours in 0,1 %; *v*: c-anaphase, 4 hours in 0,025 % chloroform. — × 1500.

chromatids and often also $^1/_4$-chromatids. Especially far-going is this morphological sub-division towards the chromosome ends, which often show signs of many threads (Fig. 1 *f*).

Certain metaphase chromosomes were considerably swelled (Fig. 1 *i*, *j*). They were very faintly stained, most of the chromatic substance already being washed out. Through the staining many fine threads were visible in longitudinal direction, clearly indicating a cable structure of the chromosomes. By the treatment evidently the many single threads, which are usually glued thickly together, had been loosened from each other. A similar division in two and more threads was present in the anaphase and telophase chromosomes (Fig. 1 *k*—*n*). The daughter half-chromatids were in these cases either turned in a relational spiral or were comparatively free from each other. Often definite chromomeres were visible, chromomeres of similar appearance occurring on the same level within the two half-chromatids.

In one slide (treated with 0,5 % for 4 hours) the dissolution of the chromosomes had often gone still one step further. Similar pictures were frequent in this slide as have earlier been found by ÖSTERGREN to occur after other narcotic treatments (unpubl.). Of the chromosomes there remained only the centromeric apparatus, the chromosome arms having completely disappeared (Fig. 1 *o*—*s*). Evidently the centromeric region is the most resistant part of the chromosomes. It was already known that this region often gives staining reactions different from the rest of the chromosomes (LEVAN, 1946, and literature given there). In the present case not only do the centromeric parts appear more darkly stained than the chromosome arms, but the latter are often totally absent. Fig. 1 *o* shows this condition in metaphasic polar view, *p*—*s* shows it from the side, at metaphase (*p*—*q*) and anaphase (*r*, *s*). It is clear that this condition gives a rare occasion to submit the centromeric apparatus to a close morphological scrutiny. Owing to the smallness of all details, however, caution must be used in the interpretation of the structures seen. It seems to be certain, however, that the centromeric apparatus is a complex structure, as has indeed been supposed by most workers in this field. The pictures show partly the heterochromatic lateral segments (the prochromosomes), partly one small body (the spindle spherule) at which the spindle fibres seem to attach. The spindle spherules at metaphase are often extended towards the poles and protrude somewhat above the surface of the chromosomes. At anaphase the spindle spherule is often the largest part of the whole centromeric apparatus present. Besides this round body, only small

irregularly shaped remnants are visible, directed from the poles (Fig. 1 *r*, *s*). The spindle spherule, on the other hand, showed about the same size in all chromosomes of one cell. No traces of the chromosome arms besides the above irregular remnants were seen.

These strong concentrations of chloroform have an immediately killing effect, the cells being fixed. A gradual dissolution takes place after the killing, which first affects the matrix, later on the whole chromosome arms, the centromeric part showing greater resistance than the rest of the chromosomes.

The next concentration, 0,25 %, is not directly lethal. The roots keep turgescent for about 4 hours, but are quite soft after 24 hours. In 0,10 % the roots remain turgescent several days. The cytological picture is here quite different. A certain general stickiness occurs, but the chromosomes are not dissolved to the same extent as in the two strongest concentrations. Furthermore, the cells keep alive long enough to show c-mitosis, which in these concentrations is the only mitosis type already occurring after four hours. A strong toxic effect is noted; after 24 hours almost only resting nuclei occur, no new mitoses being started.

C-mitosis goes down to the concentrations 0,05 and in solitary cells to 0,025 %, dubious tendencies to c-mitosis may be found even in 0,010 %. In these concentrations, however, normal mitoses predominate. The c-mitosis is of quite the ordinary type. Often the type of »bow-metaphase» (LEVAN, 1946) occurs (Fig. 1 *t*), which is characterized by the chromosomes being scattered widely over the cell, each chromosome showing little contraction, its chromatids being tightly glued together, the entire chromosomes often being somewhat arched. Especially in the few mitoses occurring after 24 hours' treatment the contraction of the c-pairs might be maximal (Fig. 1 *u*), each chromosome approaching the spherical shape. The c-anaphases (Fig. 1 *v*) were typical.

Clear c-tumours were found only in two concentrations, 0,05 and 0,025 %, the higher ones evidently being too poisonous to allow c-tumour growth. Weak tendencies to c-tumours were also seen in 0,010 %, but only in some roots.

Our results on the chloroform effect in *Allium* may be summarized as follows:

Concentrations		Roots	Dissolution of	C-reactions	
%	mol/l	turgescent	chromosomes	mitosis	tumours
1	0,08	—	+	fixing	—
0,5	0,04	—	+	fixing	—

Concentrations %	Concentrations mol/l	Roots turgescent	Dissolution of chromosomes	C-reactions mitosis	C-reactions tumours
0,25	0,02	—	sticky	+	—
0,10	0,008	+	sticky	$\frac{+}{(-)}$	—
0,05	0,004	+	—	±	+
0,025	0,002	+	—	$\frac{(+)}{-}$	+
0,010	0,0003	+	—	—	$\frac{(+)}{-}$
0,005	0,0001	+	—	—	—

III. THE EFFECT OF COLCHICINE WITH AND WITHOUT CHLOROFORM.

On indicating the concentration of colchicine solutions used in most earlier investigations a certain weight percentage has been given; no regard has been taken to whether the colchicine was pure or contained chloroform. If molar concentrations have been used, 399,32 has been employed as molar weight of colchicine, which is correct only for the pure amorphous colchicine. It is therefore of the greatest significance to test the critical threshold region within both types of colchicine in order to investigate whether differences occur which may necessitate an adjustment of threshold values earlier given. As mentioned in the introduction, some earlier studies suggest that no great differences in effect are present in the low concentrations under consideration here. Thus, LEVAN (1938), probably using amorphous colchicine, found the threshold value of c-mitosis in *Allium* to lie between 0,005 and 0,01 %. LEVAN and ÖSTERGREN (1943), with crystalline colchicine, determined the same value to be 0,004—0,006 %.

In the present experiment the following substances were used: (1) Colchicin. puriss. amorphous Sandoz, Basle, and (2) Colchicin. puriss. cryst. + $^1/_2$ mol $CHCl_3$ Merck, Darmstadt. The colchicine substances were tested for chloroform according to the isonitril-reaction (Fifth Swiss Pharmacopoeia): 2 cg colchicine, 2 cc 2-normal NaOH and 1 drop aniline are heated together. If chloroform is present the typical smell of phenyl isonitril is noticed. Colchicine (1) turned out to be completely free from chloroform, while (2) gave a strongly positive reaction. Of both substances a series from 0,002 to 0,02 % by weight were tested. Fixations of the root-tips were made 4 and 24 hours after starting the treatment. The results as far as c-mitosis and c-tumours are concerned are given in Table 1.

From this table it is seen that both series behave perfectly alike.

TABLE 1. *Comparison between different forms of colchicine.*

Weight %	Amorphous colchicine ($C_{22}H_{25}NO_6$)				Crystalline colchicine ($C_{22}H_{25}NO_6 + {}^1/_2\ CHCl_3$)			
	Molarity of colchicine	c-mitosis		c-tumours	Molarity of colchicine	c-mitosis		c-tumours
		4 hours	24 hours			4 hours	24 hours	
0,02	0,00050	+	+	++	0,00043	+	+	++
0,01	0,00025	+	+	+	0,00022	+	+	+
0,007	0,000175	±	±	+	0,00015	+	±	+
0,004	0,000100	±	±	±	0,000087	±	±	±
0,002	0,000050	—	—	—	0,000043	—	—	—

The agreement is further accentuated by Fig. 2, which shows the c-tumour reaction of the two series. Not only are they alike in the general type of the reaction but also in the smallest details. The tumours of 0,02 % are, for instance, of similar size and shape in both

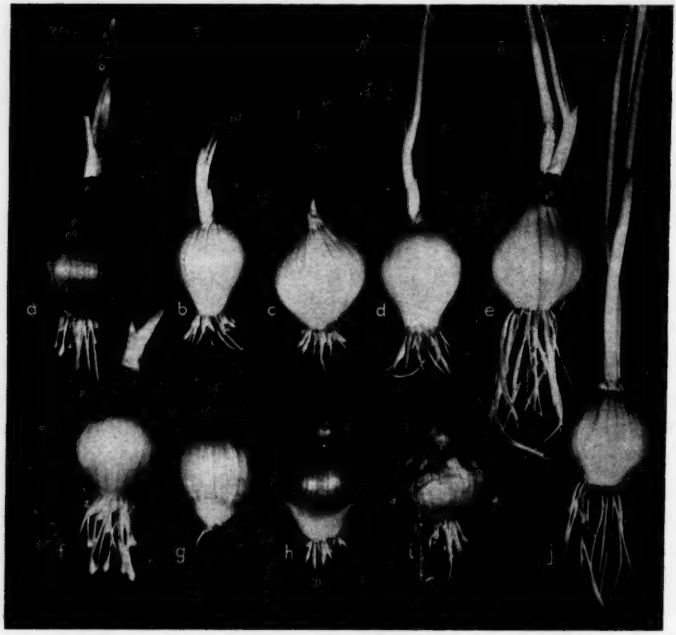

Fig. 2. The c-tumour reaction in chloroform-containing colchicine (*a—e*) and in pure colchicine (*f—j*); *a*, *f*: 0,02 %, *b*, *g*: 0,01 %, *c*, *h*: 0,007 %, *d*, *i*: 0,004 %, *e*, *j*: 0,002 %.

series, and are somewhat distinguished from the tumours of the lower concentrations. The limit concentration 0,004 % in both series starts typical tumour growth, but after some time tumours are overgrown by normally elongating root-tips.

The experiment has shown that in this concentration range it is quite without consequence which form of colchicine is used. Earlier determined threshold values may thus be relied upon independently of what kind of colchicine has been used.

IV. CONCLUSIONS.

The above experiments have shown that chloroform gives typical c-mitotic and c-tumour reaction in concentrations down to 0,025 % by weight (0,002 mol/l). In crystalline colchicine, where chloroform is present in fractions of $^1/_4$ or $^1/_8$, concentrations of 0,1 or 0,2 % would be needed to reach the threshold value of chloroform. In the concentration range of colchicine giving c-mitosis in *Allium*, 0,002—0,004 %, there is evidently no risk that the chloroform will have any c-mitotic effect. The small deficit of colchicine in the crystalline form is not sufficient to exceed the limits of normal experimental error.

The thermodynamic activity of chloroform may be determined according to our results; it is calculated as the relation of threshold value to water solubility. As a comparison the same values for ether are given, the threshold for c-mitotic action being taken from ÖSTERGREN (1944):

Substance	Solubility in 10^{-6}mol/l	Threshold in 10^{-6}mol/l	Thermodynamic activity
Chloroform	83'000	800—2'000	0,01—0,02
Ether	1'000'000	62'500—125'000	0,06—0,13

A thermodynamic activity of 0,05 is considered to denote the extreme lower limit for a pure unspecific, physical activity of a narcotic (FERGUSON, 1939; GAVAUDAN, DODÉ and POUSSEL, 1945). Since chloroform evidently goes below this limit in our experiments, a certain degree of chemical activity can hardly be denied in this substance. The activity of ether, on the other hand, is well within the limits of physical activity. In this connection it should be pointed out that chloroform undoubtedly had a definite toxic effect on the *Allium* roots as far down as our series went, i. e. to 0,0004 mol. Thus, the mitotic activity was almost completely inhibited after 24 hours in this concentration. It was also noticed that

a weak poison effect was present in some of the tested concentrations of crystalline colchicine which was not present in the amorphous substance. It should be mentioned that chloroform, in contradistinction to ether, is known for a considerable toxicity in its narcotic action on animals. This condition has actually limited its usefulness as an anaesthetic in surgical practice.

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SUMMARY.

The cytological effect of chloroform as well as of amorphous and crystalline colchicine is studied. Strong concentrations (saturated—$^1/_2$ saturated solutions) of chloroform bring about a characteristic cytological effect. The chromosomes are dissolved, in extreme cases only the centromeric apparatus is left. In lower concentrations the c-mitotic reaction is found. The threshold for this reaction is 0,0008—0,002 mol/l. The threshold region of c-mitosis in *Allium* in the two types of colchicine was found to coincide, the chloroform present in the crystalline colchicine being too diluted to have any c-mitotic effect. The water solubilities of the substances employed here are discussed and the results of our experiments are put in relation to the thermodynamic activity of the threshold concentrations.

Svalöf, March, 1947.

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CONTENTS.

I. Introduction 515
II. The effect of chloroform 517
III. The effect of colchicine with and without chloroform 521
IV. Conclusions 523
Summary 524
Literature cited 524

Communications from the Swedish Seed Association, No. 3.

SOME OBSERVATIONS ON THE SEED DEVELOPMENT IN ECUADORIAN CACAO

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PROFESSOR ARNE MÜNTZING has made observations and experiments on pollination and fruit setting in cacao trees at the Swedish Hacienda Clementina in the neighbourhood of Babahoyo in Ecuador (MÜNTZING, 1947). A number of ovaries and young fruits (cherelles) from hand-pollinated flowers were fixed for embryological studies and thus I have been able to study some stages of the seed development in this material. The development of the embryo-sac in *Theobroma cacao* has been studied by KUYJPER (1914); on fertilization and later stages of the seed development there is a good investigation by CHEESMAN (1927). The embryo-sac develops after the normal type, and is rather small. In ovaries fixed five or six hours after pollination pollen tubes could be observed in the micropylar region of the embryo-sac, and two hours later CHEESMAN could sometimes observe male nuclei lying against the egg or the polar nuclei. »The first division in the embryo-sac takes place sometimes on the third but more often on the fourth day after fertilization, and in preparations made then two endosperm nuclei are found» (CHEESMAN, l. c.). Fig. 12 of CHEESMAN shows the second division of the endosperm nucleus 120 hours after pollination. The first cell walls in the endosperm appeared when the fruit was not less than 50 days old. Very slow is the development of the embryo, the youngest embryo observed was in a fruit 50 days old. »The first division of the fertilized egg was not observed, but apparently it occurs from forty to fifty days after fertilization.» CHEESMAN found in nearly full-grown fruits that the embryo may be less than two mm. long, but later the embryo must grow very rapidly, »its development being nearly complete when the fruit stops growing». Parthenocarpy does not occur.

The literature on pollination, fertility and fruit development in cacao is rich (compare MÜNTZING, l. c.). It is a well known fact that the cacao tree produces a very large number of flowers (50.000), of these a varying number set (in one case almost 6.000), while perhaps 100 mature fruits are a good crop. Thus most ovaries are dropped the first week after the flowering, and during some following weeks (3—7)

a variable number of developing pods wilt, the so-called cherelle wilting. The fruit is mature after about 6 months. POUND (1931 a) has observed a constricted region on the pedicel, where the abscission of the flower is brought about by an active osmotic mechanism. POUND thinks that the rapid growth of fertilized ovaries drains the soluble carbohydrates of the pedicel and therefore the osmotic mechanism does not function and the flower is not dropped. There is no self-pollination within the cacao flower. Pollination has been claimed to be effected through the agency of thrips, aphids and red ants. However, the Ceratopogonid midge *Forcipomyia* has been shown to be responsible for most of the effective pollination of cacao flowers in Trinidad (see POSNETTE, 1944). At Clementina aphids, directly or indirectly through ants, contribute to pollination; the spontaneous pollination was, however, very often faulty, many styles having no or only a few pollen grains (MÜNTZING, l. c.). POUND (1931 b) has shown that ovaries from different trees contain about the same number of ovules, while mature fruits have a varying number of beans, 25 % or as many as 50 % of the ovules not having developed into beans. Later (POUND, 1934) it was shown that ovaries six days after pollination may have a varying number of ovules that had not increased in size, these ovules probably being unfertilized. The variation of the bean number in mature fruits is thus largely a result of incomplete pollination, and MÜNTZING finds that this agrees with the conclusions drawn from his own observations. POUND (1931 a) has further shown that the cherelle wilting cannot be due to incomplete fertilization, because an investigation of cherelles having a length of two inches showed that the number of good ovules was about the same in wilted fruits as in good fruits (the cherelle wilting has been attributed to a competition for water and nutrients).

In Trinitario and Criollo cacao there are self-compatible (self-fertile) and self-incompatible (self-sterile) trees. The latter have no or very reduced fruit setting when self-pollinated or pollinated with pollen from other self-incompatible trees; they have good setting when pollinated with pollen from self-compatible trees (see POSNETTE, 1944). COPE (1938) has shown that the incompatibility is not due to an arrested or reduced growth of the pollen tubes. POSNETTE showed that incompatible pollen on the stigma caused reduced setting when mixed with compatible pollen unless the latter had some hours better start or a shorter tube-path to travel. POSNETTE (1945) showed through hand-pollinations that self-incompatible types are very common in Amazonas cacao, although they differ from self-incompatible Trinitario types in

being cross-compatible. MÜNTZING (l. c.) used four trees for his pollination experiments, the trees 2, 3 and 4 were self-incompatible while tree 5 was self-compatible. Cross combinations resulted in a commencing fruit development in the cross self-incompatible × self-compatible (3 × 5 and 5 × 3), and in one of the crosses self-incompatible × self-incompatible (2 × 3 and 3 × 2), whereas 3 × 4 was quite negative.

An embryological investigation on compatible and incompatible cacao crosses has already been made by COPE (1939). Flowers of a self-incompatible tree were selfed or pollinated with pollen from a self-compatible tree; fixations were made, the latest 96 hours after pollination. Fertilization was the same in ovaries pollinated incompatibly as in those pollinated compatibly; after 30 hours no difference could be observed. Certain post-fertilization differences became, however, more pronounced with lapse of time. Division of polar nucleus was initiated considerably later in incompatibly fertilized embryo-sacs, and at abscission less than 25 % of the embryo-sacs showed divided polar nuclei. While in the compatible cross after 58 hours 22 % of the embryo-sacs had divided polar nuclei, after 72 hours 82 %, the corresponding figures from the incompatible cross were 0 % and 5 %. COPE considers that the late inception and low level of nuclear activity in incompatibly pollinated ovaries are responsible for abscission. POSNETTE (1945) showed that in ovaries of a self-incompatible Amazonas type pollen tubes had entered the embryo-sacs 30 hours after pollination, thus confirming the observations of COPE.

The results from the investigation of the Clementina material were the following. It must be observed that in most cases the content of all ovules in the ovaries (their number is more than forty) could not be studied, because many sections were often lost during the staining of the slides and the starch grains in the embryo-sac often obscured the polar nuclei, making observations on their behaviour impossible.

Selfing of 5 (compatible pollination). — 10 hours: in one ovary 17 fertilized, 2 unfertilized embryo-sacs were counted, in one 5 fertilized, 12 unfertilized, in one 2 fertilized, 12 unfertilized. In »unfertilized embryo-sacs» both the synergids were intact and no trace of the pollen tube could be detected in the sac. — 24 hours: in two ovaries most embryo-sacs were fertilized, in one all the embryo-sacs were unfertilized. In the latter case the ovules and embryo-sacs had a rather youngish appearance. — 48 hours: two ovaries had very few, one had rather many, unfertilized embryo-sacs. In unfertilized embryo-sacs the polar nuclei are still in the vicinity of the egg-apparatus, in fertilized embryo-sacs they have moved and are often near the chalaza, and one of them often has two nucleoli suggesting fusion

with a sperm. — 72 hours: only one fruit could be investigated. The great majority of the embryo-sacs were fertilized, and in such embryo-sacs the polar nuclei were often united to a central nucleus; several had, however, polar nuclei. The division of the central nucleus (meta-, ana- and telo-phase) was observed only three times (a number of sections had been lost). — 7 × 24 hours: two ovaries had probably only enlarged (that is fertilized) ovules, two had many not enlarged ovules. The latter were unfertilized, the former had two or mostly four endosperm nuclei. — 8 × 24 hours: in three fruits most ovules, in one about half the number of ovules, in one no ovule was enlarged. The fruit last mentioned was very small. Enlarged ovules had four or eight endosperm nuclei, or four nuclei dividing. — 12 × 24 hours: in one fruit less than half the number of the ovules were enlarged, in other fruits the great majority were enlarged. Enlarged ovules had in most cases eight, rarely four or sixteen, once only two endosperm nuclei. — A varying number of mitoses could be observed in the somatic tissue of the developing ovules in all fixations.

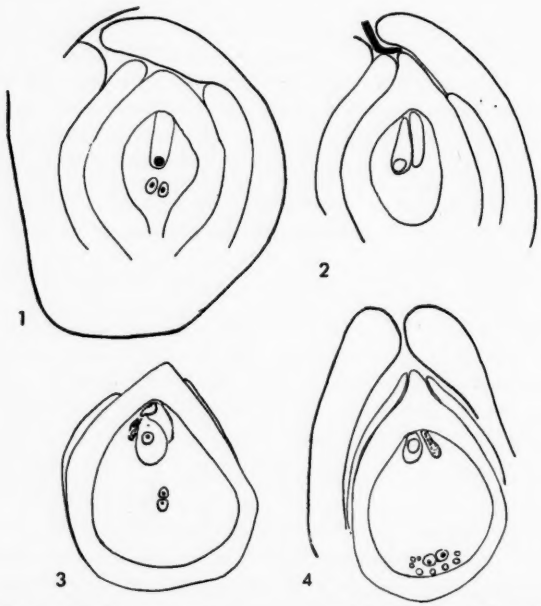
Selfing of 3 (incompatible pollination). — 10 hours: in each of seven ovaries investigated only a few ovules were fertilized. — 25 hours: most embryo-sacs were fertilized, polar nuclei were sometimes of different size, indicating fertilization. — 48 hours: in one ovary were 31 fertilized, 3 unfertilized, in one 30 fertilized, 1 unfertilized, in one 13 fertilized, 24 unfertilized embryo-sacs. The polar nuclei were moving towards the chalaza. — 72 hours: four fruits were investigated, as a rule the embryo-sacs were fertilized. Only rarely a central nucleus was formed. The polar nuclei in fertilized embryo-sacs were as a rule near the chalaza and showed signs of being fertilized. Sometimes the pollen tube had disorganized a synergid, the egg-cell and polar nuclei were, however, not fertilized and no sperms were observed. Two embryo-sacs showed the division of the central nucleus, and two contained two endosperm nuclei. — Mitoses were rare in the ovules after 72 hours, but numerous after 48 hours.

3 × 5 and 5 × 3 (compatible pollinations). — 10 hours: in 3 × 5 one ovary contained only fertilized embryo-sacs, one only unfertilized, while a third had about half the number of its embryo-sacs fertilized. Similar conditions were shown by 5 × 3. — 96 hours: in 3 × 5 three fruits were relatively large and had most ovules fertilized, one fruit was rather small and had only about half the number of ovules fertilized, in another small fruit all ovules were unfertilized. Fertilized embryo-sacs had in most cases two endosperm nuclei, in some cases the division of the central nucleus was observed, the central nucleus was rarely undivided. In 5 × 3 fertilized embryo-sacs had two or four endosperm nuclei, in one case, however, polar nuclei. — 7 × 24 hours: in 3 × 5 the enlarged ovules in most cases had four (sometimes two or eight) endosperm nuclei, in 5 × 3 the most common number of endosperm nuclei was eight. — Mitoses in the fertilized ovules of some fruits were common, in other fruits more rare.

2 × 3 and 3 × 2 (probably compatible pollinations). — 96 hours (3 × 2): in one ovary most ovules were fertilized, in one only few were fertilized, in one about half the number were fertilized. Fertilized ovules had two or four endosperm nuclei. — 7 × 24 hours (2 × 3): enlarged ovules had in most cases four, sometimes only two, endosperm nuclei (the occurrence of eight was doubtful). — 7 × 24 hours, a wilted cherelle (2 × 3): about half the number of ovules were enlarged with in most cases four endosperm nuclei, the egg-cells were indistinct. The inner integument had degenerated in the unfertilized ovules, but this was also the case

in unfertilized ovules of the non-wilted cherelle. — 10 × 24 hours (2 × 3): enlarged ovules have in most cases eight endosperm nuclei. — 8 × 24 hours (3 × 2): most ovules were enlarged. They had eight or more rarely four endosperm nuclei. A few of the ovules that had not been enlarged showed indication of a fertilization of the embryo-sac, however, their polar nuclei were not divided (Fig. 4). — 12 × 24 hours (3 × 2): some ovules were degenerated. Enlarged ovules had mostly eight endosperm nuclei.

3 × 4 and 4 × 3 (probably incompatible pollinations). — 72 hours (3 × 4):



Figs. 1—4, ovules of *Theobroma cacao*. — *1*: 5 selfed, 48 hours after pollination. — *2*: 3 × 5, 25 hours after pollination, pollen tube in the micropylar canal. — *3*: 4 × 3, 96 hours after pollination, the embryo-sac was probably not fertilized, the inner integument is collapsed; the outer integument is not figured. — *4*: 2 × 3, 8 days after pollination, the pollen tube had penetrated into the embryo-sac but the polar nuclei are probably not fertilized; they are still surrounded by starch. — × 230.

in the ovary investigated most embryo-sacs were fertilized. They had polar nuclei or a central nucleus, which often were not near the chalaza, rarely two endosperm nuclei. — 4 × 24 hours, a dropped flower (4 × 3): one fruit, about half the number of embryo-sacs were fertilized. Such embryo-sacs had two, but in some cases four, endosperm nuclei (Fig. 14). In several embryo-sacs having a disorganized synergid polar nuclei had not been fertilized. — Mitoses were rather common in the inner integument after 72 hours, but could very rarely be observed in the dropped flower. In the latter, unfertilized ovules were smaller than fertilized and their inner integu-

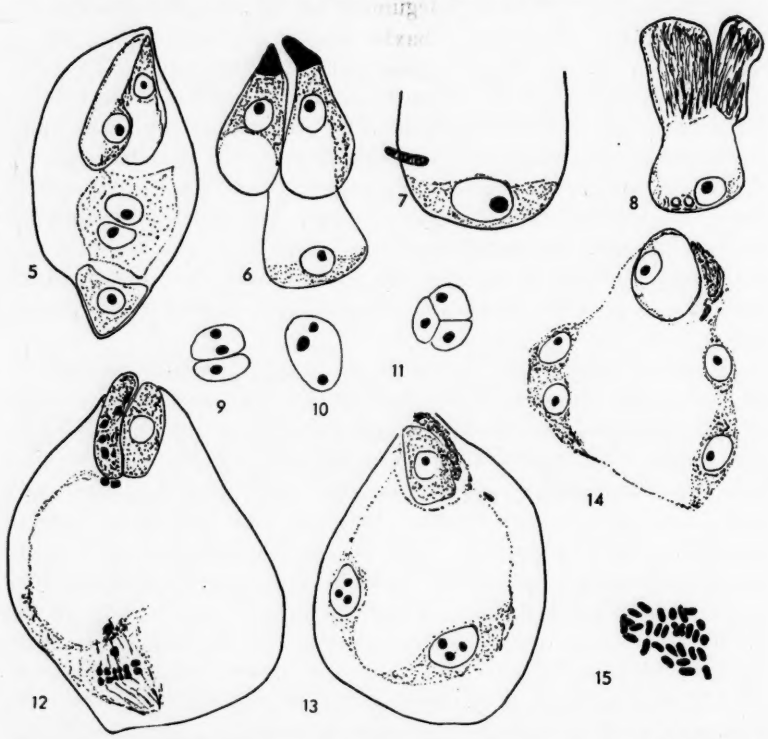
ment was often degenerated (Fig. 3). The egg-cell was distinct in most embryo-sacs of the dropped ovary.

From the observations made a general description of the seed development, which in some points supplements the papers of CHEESMAN and COPE, may deserve publication. The outer integument is higher than the inner integument; it is not, however, developed on the funicular side of the ovule. The inner integument has its strongest development on the funicular side, on the abaxial side of the ovule its growth is arrested before it reaches the apical part of the nucellus. Thus the micropylar canal, which is directed laterally towards the placenta, is on the funicular side limited by the inner integument, while the outer integument is in contact with the apical part of the nucellus (Fig. 1), the zone of contact sometimes being rather broad (Fig. 2). The pollen tube grows through the micropylar canal to the apex of the nucellus and then through the nucellar layers to the embryo-sac. The tube was more easily observed at the entrance of the micropylar canal or in the nucellus in 3 or 3×5 than in 5 owing to the fact that it was thicker here (Fig. 2).

Only very rarely was the embryo-sac aborted. Organized embryo-sacs had a very large egg-apparatus (Fig. 5). The synergids are rather variable; the basal part of the synergid cells is sometimes a rather long cone, but in other embryo-sacs it is broad with conspicuous synergidal lists. Sometimes each synergid had a cap (Fig. 6); in slides stained with light-green the caps were green; they are thus probably made of cellulosa, as was expected. After the pollen tube has entered the embryo-sac one synergid is disorganized. The egg-cell is long and poor in cytoplasm. Fertilized egg-cells undergo certain changes, they become shortened, are relatively richer in cytoplasm and their nucleus is larger. They enter, as has been shown by CHEESMAN, into a long resting stage: after 12 days no egg-cell was divided in the Clementina material. The polar nuclei are in the vicinity of the egg-apparatus, after fertilization they move to a position near the chalaza, where the first division of the endosperm occurs (Fig. 12). In 5 they had more often than in 3 reached the chalaza after 48 hours. If fertilization of the embryo-sac fails, they as a rule remain in their original position, but are perhaps able to move later: a week after pollination they have sometimes been observed near the chalaza in embryo-sacs that were probably unfertilized. The antipodal cells of the embryo-sac are not particularly small but are ephemeral. The starch grains in the embryo-sac have been described and figured by CHEESMAN and COPE. They are ofter clustered

on the polar nuclei and follow these to the chalaza. After 72 hours they tend to be more dispersed, diminish in number and have often disappeared after 96 hours. In unfertilized embryo-sacs they remain longer.

CHEESMAN and COPE have pictured a small male nucleus in contact with the egg nucleus. CHEESMAN also figures a small male nucleus in



Figs. 5—15, *Theobroma cacao*. — *5*: embryo-sac of 5; one synergid and two antipodals are not figured. × 760. — *6*: egg-apparatus of 5, the synergids have caps. × 920. — *7*: 3 × 5, 25 hours after pollination, sperm on the side of the egg-cell. × 1900. — *8*: 5 selfed, 24 hours after pollination; two male nuclei in the egg-cell. × 920. — *9*: 5 selfed, 72 hours after pollination; polar nuclei, one of them fertilized. × 760. — *10*: 5 selfed, 72 hours after pollination; central nucleus. × 760. — *11*: 3 selfed, 48 hours after pollination; polar nuclei and male nucleus. × 760. — *12*: 3 selfed, 72 hours after pollination; the first division of the endosperm. × 760. — *13*: 3 selfed, 72 hours after pollination; the embryo-sac has two endosperm nuclei. × 760. — *14*: 4 × 3, 96 hours after pollination; four endosperm nuclei. × 760. — *15*: nuclear plate — the endosperm divisions; the number of chromosomes is (about) 30. × 1900.

contact with the two polar nuclei. I have found observations on fertilization difficult. The male nuclei seem to change form and size when introduced into the embryo-sac. Fig. 7 shows a spindle-like chromophilic sperm from the vicinity of the egg-cell. Later the sperm seems to have taken the appearance of an ordinary nucleus as in the figures of CHEESMAN and COPE; the egg-cell in Fig. 8 seemed to contain two such nuclei. Fig. 11 indicates that the male nucleus may approach the size of a polar nucleus. However, in the Clementina material triple fusion only rarely occurs. In fertilized embryo-sacs the polar nuclei were of different size and one of them had two nucleoli (Fig. 9). Thus the male nucleus in most cases fertilizes one of the polar nuclei; later the polar nuclei unite, forming a central nucleus having several nucleoli (Fig. 10). A central nucleus was only observed after 72 hours, and thus is formed immediately before the first division of the endosperm. In only a minority of the embryo-sacs of 5 did this division occur at 72 hours, and thus it is distinctly later than in the compatible combination studied by COPE and about as late as in the material of CHEESMAN. After 96 hours the fertilized embryo-sacs as a rule had two (or more rarely four) endosperm nuclei; undivided polar nuclei were then rare. This was also the case in the probably incompatible combination 4×3, and here the more sluggish development found by COPE after incompatible pollination perhaps did not occur. The latest fixation made of the incompatible 3 was after 72 hours. In some ovules the first division in the endosperm was observed or had occurred, but, as many more ovules could be studied of 3 than of 5 while the number of observed divisions was about the same in the two cases, it seems that the division is retarded in the incompatible combination. And it is a fact that a central nucleus could only seldom be observed in 3, the polar nuclei being rarely united after 72 hours. Thus selfing of 3 seems to result in a belated fertilization and a retarded nuclear activity in the fertilized embryo-sac.

The first endosperm nuclei are relatively large and have several nucleoli. The size of the endosperm nuclei diminishes in the following divisions. The chromosome number of the endosperm was as expected 30 (Fig. 15). The rate of development of the endosperm is slow. There seemed to be differences of this rate in the two reciprocal crosses with 3 and 5, but the investigated material is obviously too small to allow of any certain conclusions in this respect. In some ovules of a cherelle the rate of development of the endosperm was retarded. There are also

differences in the size of the embryo-sacs in the same cherelle, some of them being conspicuously broader or smaller than the majority.

The inner integument has two or three layers at the time of fertilization. In all fixations of 5 one observes cell-divisions in the inner integument and nucellus of the fertilized ovules; if fertilization fails the divisions cease. Many mitotic spindles have a radial orientation and therefore the bulk of the nucellus and the number of layers of the inner integument increase, the latter to 4—5 after a week and 5—6 after 12 days. After the probably compatible crosses between 2 and 3, mitotic divisions seemed as a rule rarer than in 5, and this is confirmed by the inner integument having only 4—5 layers after 10 days, 4 layers after a week. After a week the inner integuments of 3×5 had 4 layers, those of 5×3, 4—5 layers. The incompatible selfing of 3 had mitosis in many ovules after 48 hours, but after 72 hours only few of the fertilized ovules had one or two divisions in the inner integument and the nucellus. In the probably incompatible combination 3×4, however, cell-divisions were numerous in many ovules after 72 hours, but very few divisions could be observed after 96 hours (in a dropped flower).

In all cherelles investigated there was at least some unfertilized ovules. Incompatible pollination seems to result in belated fertilizations, but after 48 hours the great majority of the ovules may be fertilized; after compatible pollinations, too, as a rule not all ovules are fertilized. The unfertilized ovules of the cacao seem to merit a description. 96 hours after pollination ovules with unfertilized embryo-sacs are distinctly smaller than fertilized ovules in the same cherelle owing to the earlier cessation of cell-division. The embryo-sac has, however, enlarged. It is quite as broad as the fertilized embryo-sacs with endosperm nuclei but shorter. During the widening of the unfertilized embryo-sac several nucellus layers are destroyed. The behaviour of starch grains and polar nuclei have been described above: the cells of the egg-apparatus soon become poor in cytoplasm, and are often indistinct. A degeneration of the somatic tissue occurs and this degeneration always begins with the inner integument. As early as 96 hours after pollination in some or most unfertilized ovules the cells of the inner integument have collapsed, and after a week the inner integument has collapsed in all unfertilized ovules. The remaining nucellus layers also collapse and, as the inner integument is mostly close to the nucellus layers, the unfertilized ovules after a week have a peculiar hollow appearance like an empty shell (Fig. 3). Then the ovules dry and the lumen of the embryo-sac shrinks; after 10 or 12

days almost all unfertilized ovules have dried. A similar appearance is assumed by ovules where a pollen tube had disorganized a synergid but the polar nuclei could not divide, probably due to failure of fertilization (Fig. 4).

Conclusions. — Embryological studies may help to an understanding of the different sterility in cacao: the early drop of many ovaries, the cherelle wilting and the aborted seeds in such pods as reach maturity. A special problem is offered by the incompatibility. The early drop after compatible crossings is probably mostly a result of failures of pollination. POUND found that unpollinated flowers remain on the tree 48 hours at the highest, owing to the function of an abscission mechanism. The material here investigated had been hand-pollinated and therefore a number of pollen grains probably sufficient to fertilize all ovules in the ovaries had been brought to the stigmas. It is, however, a fact that as a rule a variable number of the ovules in these hand-pollinated flowers were unfertilized. In certain extreme cases no ovule was fertilized, and such ovaries could remain on the tree a week. This is perhaps an indication that pollination *per se*, that is pollination not followed by fertilization, tends to retard the function of the abscission mechanism. Cherelles with no fertilized ovules were much smaller than normal cherelles of the same age; an intermediate size had cherelles where about half the number of ovules were fertilized. That the abortion of seeds in the pods to a large extent depends on failures of fertilizations is quite clear; in a cherelle 12 days old no doubt half the ovules could be unfertilized. In some cases the ovule was fertilized but development did not start. There were also a certain though low number of fertilized ovules that were unusually small and had a narrow embryo-sac with an abnormally low number of endosperm nuclei. Such ovules probably degenerate later, having too low a rate of development, and may thus increase the seed sterility. A fourth cause of seed abortion may perhaps be degeneration of the egg-cell or the embryo. Such abortions could not be studied and it is unknown whether they occur in any appreciable frequency.

Concerning the question of cherelle wilting, it has already been mentioned that POUND found the number of good ovules to be about the same in good and wilting cherelles. However, an embryological investigation has not been made; many ovules in the wilting cherelles are perhaps abnormal. Professor MÜNTZING was only able to make fixations during 12 days, and thus the cherelle wilting could not be studied here. BRINK and COOPER (1941) have shown (their paper has also been cited

by MÜNTZING) that in certain species hybrids there are a slow development of the endosperm and an excessive growth of the somatic tissue of the ovule (integument or nucellus), leading to starvation and death of the embryo. This kind of sterility they call somatoplastic sterility, and they consider the abnormality of the somatic tissue to be a cause of the slow development of the endosperm, and this in its turn leads to the abortion of the embryo and the seed. After a review of the surprisingly meagre literature on the embryology of dropped orchard fruits they express the opinion that the so-called June drop of orchard fruits is a result of abortion of seeds, this seed abortion being caused by somatoplastic sterility. It may be mentioned that LUCKWILL (1946), in apple seeds from three to ten weeks old, found a fruit setting hormone. The fructigenic activity of the seeds expires about 10 weeks after the petal fall, that is at the time of the June drop.

It is tempting to compare the cherelle wilting of cacao with the June drop of orchard fruits, somatoplastic sterility perhaps causing a weak development of the seeds and thus an insufficient production of a fruit setting hormone. However, one feature of somatoplastic sterility, excessive development of nucellus and integument, one would hardly expect to find here. CHEESMAN showed that there is »a considerable development of the nucellus, forming perisperm, whilst up to the time of wall-formation the endosperm is a very thin layer lining the embryo-sac. From this point onwards the endosperm increases more rapidly, and the perisperm is soon reduced in proportion». The first wall-formation in the endosperm was observed by CHEESMAN in fruits about fifty days old. As normally the development of nucellus is strong and of endosperm weak, it seems unlikely that excessive development of nucellus can be a cause of seed abortion in cacao. However, the developing endosperm may perhaps sometimes have inherent weakness leading to abortion, the endosperm not being able to reduce the perisperm. An embryological investigation of the state of the seeds in good and wilting cherelles is, however, needed.

The incompatibility in cacao is not a result of failure of fertilization, as has been shown by COPE, who, however, found the development in the embryo-sac to be more sluggish after incompatible pollinations. The delayed and slow development of the embryo-sac after incompatible pollinations is responsible for the failure to set fruit and the abscission. »Where, as in compatibly pollinated ovaries, activity of the contents of the embryo-sac is at a high level of intensity, then the turgor abscission mechanism does not operate.» COPE had investigated

cacao from Trinidad. POSNETTE concludes concerning the self-incompatibility in Amazonas cacao (here crosses between incompatibles are compatible): »It is tentatively suggested that the failure to inhibit the maternal mechanism of flower-shedding occurs when the pollen tube carries the same incompatibility gene as the maternal tissue» (1945). Of the two incompatible combinations investigated here, 3×3 and 3×4, the former showed a slower development in the fertilized embryo-sacs than compatible combinations, though the difference does not seem to be as great as that found by COPE. I could not observe that »some sacs were completely devoid of nuclear content, although starch persisted» (COPE, l. c.), such a state of things having only been observed by me in older unfertilized embryo-sacs. There was less evidence of sluggish activity in fertilized embryo-sacs of the combinations 4×3; as a rule fertilized ovules had two or four endosperm nuclei after 96 hours.

The observations made here indicate that after incompatible pollinations fertilized ovules soon show no divisions in nucellus and the inner integument. After 72 hours only a few ovules had some cell-divisions in 3×3, and in 3×4 they had ceased after 96 hours even if the embryo-sac of the ovule had endosperm nuclei and therefore had shown normal activity. If fertilization fails, the cell-divisions in the somatic tissue of the ovule stop and the cells of the inner integument soon collapse, the degeneration being first manifest in this tissue. Fertilization starts a new nuclear activity in the somatic tissue of the ovule, mitoses in nucellus and inner integument soon becoming numerous, but it seems that the stimulus to new nuclear activity is weak after an incompatible fertilization. This cessation of mitosis was evident in all ovules of the ovary that had been pollinated incompatibly even if they had an embryo-sac with four endosperm nuclei, and thus perhaps the fertilization (of the polar nuclei?) has a direct influence on the somatic tissue. After incompatible fertilizations this influence is perhaps too weak; there is soon no nuclear activity in the somatic tissue and therefore the function of the abscission mechanism is not stopped.

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DISTURBANCES IN MICROSPORE CYTOLOGY OF ANTHOXANTHUM

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IN 1935 GEITLER showed that the microspore is a polarized cell in which the axis of the division of the nucleus of the pollen grain is constant and characteristic of each species or genus.

The development of the microspore in *Tradescantia* has been carefully described by SAX and EDMONDS (1933). From the description of these authors we can recognize three steps in the development of the pollen grain before the second pollen mitosis: firstly, the movement of the nucleus to a special position accompanied by changes in the cytoplasm and movements of the vacuole; secondly, the orientated division; and, thirdly, the differentiation of the two new nuclei. The second pollen division involving only the generative nucleus follows in the pollen tube during the germination of the grain.

In *Anthoxanthum*, as in other grasses, the first division of the pollen grain has a characteristically oriented and asymmetric spindle and it gives rise to two nuclei of different shape and appearance: one darkly stained that originates near the cell-wall, and another faintly stained larger and more rounded that originates in the middle of the cell. The second division also takes place inside the pollen grain, it consists in the division of the more stained nucleus called the generative, while the other, the vegetative, becomes more and more pale, and remains a resting nucleus. In this division of the generative nucleus, the chromosomes are much contracted, and after telophase the two daughter nuclei start to assume spindle shape.

In the material of wild origin of *Anthoxanthum aristatum* BOISS., with B chromosomes, kindly ceded by Dr. G. ÖSTERGREN (ÖSTERGREN, 1947), there were found some disturbances in this process.

It was observed that in 7 plants with different numbers of B chromosomes the disturbances were as listed in Table 1.

Non-orientation, weak or non-differentiation of the two nuclei. — In all the plants, besides the pollen grains with normal orientation and differentiation, there could be found as an occasional intermixture, varying from anther to anther, such in which the two nuclei were un-

TABLE 1. *Disturbances in the microspores of A. aristatum.*

Reference number	Number of B chromosomes	Disturbances observed		
		Non-orientation, weak or non-differentiation of the 2 nuclei	Supernumerary mitosis	Chromatin drops
1	1			
2	1	»	—	—
3	1	»	—	—
4	2	»	—	—
5	2	»	—	—
6	2	»	—	—
7	4	»	—	—

distinguishable from one another or where they presented all degrees of transition between complete differentiation and no kind of differentiation (Figs. 19 and 20). This non-differentiation was accompanied by an absence of orientation and the nuclear development was in most cases displaced in the direction of a weaker staining or less commonly in the direction of a stronger staining, that is to say, the two nuclei approximated the appearance of a vegetative nucleus or in a few cases (plant 2) that of a generative nucleus.

In plants 2 and 7 there could also be found, besides these non-differentiated grains, other abnormal pollen grains with degenerating nuclei and empty pollen grains. These last ones have been considered as the final result of the degeneration of those non-differentiated pollen grains which have been unable to proceed to division and life. This view was supported by the fact that in the same anther, besides non-differentiated pollen grains, there were many pollen grains with three well differentiated nuclei (with two sperm nuclei already of spindle shape). This shows that these non-differentiated nuclei really could not reach division, or were much delayed in attaining it.

In another plant (plant 1) non-differentiation was not only followed by degeneration but also by supernumerary mitosis; the two nuclei were able to divide and they sometimes reached the stage of 4 nuclei.

Supernumerary mitosis, chromosome contraction and spindle disturbances. — In plant 1 it was found that the pollen grains in some cases develop with a normal differentiation and orientation of their nuclei, which then also divide normally, but in other cases there results

a non-orientation and weak differentiation connected with division of one or both the two nuclei formed after the first division. It has been possible to recognize a complete series of transitions between, on one hand, the division of only the generative nucleus and non-division of the vegetative nucleus (the normal case) and, on the other hand, the complete division of the vegetative nucleus without the division of the generative.

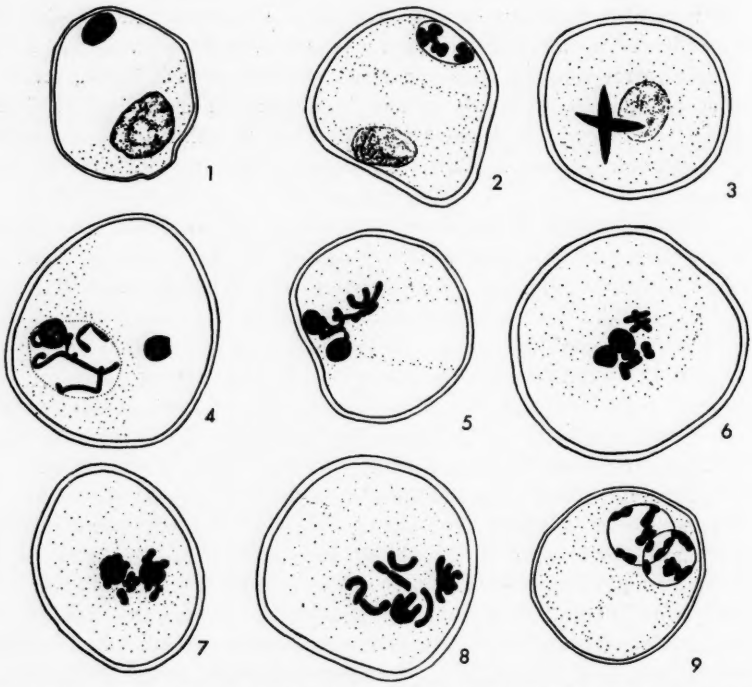
Looking at Figs. 1 to 3 we can see the normal case (case 1) in which the vegetative nucleus remains palely stained and the generative (Fig. 1) begins to divide (Fig. 2), giving origin to the 2 spindle-shaped sperm nuclei (Fig. 3). The chromosomes appear (Fig. 2) already contracted.

In the second case, after the normal division of the generative nucleus, the vegetative begins to divide. In Fig. 4 a prophase of the vegetative nucleus with the five chromosomes is seen between two typical sperm nuclei; in Fig. 5 a normal metaphase plate in the middle of two sperm nuclei; in Fig. 6 a metaphase in which the chromosomes are just divided and much contracted, with the appearance of a colchicine-mitosis; in Fig. 7 the case of an abnormal anaphase in which the pairs of chromosomes can still be seen, coming most probably from an abnormal metaphase like that of Fig. 6. Figures very similar to these with c-mitotic appearance and extra contraction have been recorded by BARBER (1941) after heat-treatment in the pollen grains of *Uvularia*, and SAX (1937 b) has also found contraction of the chromosomes in *Tradescantia* (a triploid plant).

The third case (Fig. 8) is considered as being most probably the result of the division of the generative nucleus immediately followed by the division of the vegetative, which is developing a metaphase plate between the two anaphase groups of the generative nucleus.

In case four (Figs. 9 and 10) a complete synchronization of the two nuclei is found and it is only possible to guess which is the vegetative and which is the generative nucleus by the number of chromosomes, but in all other respects they are undistinguishable.

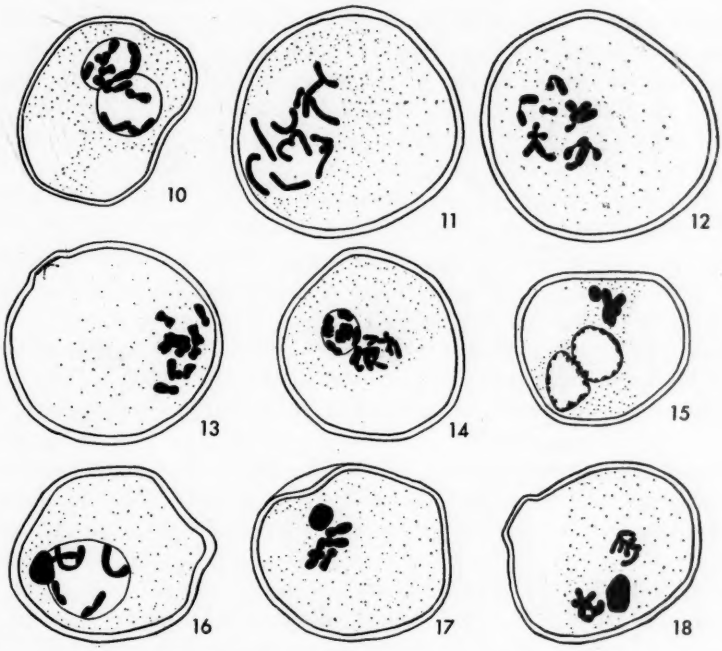
In *Anthoxanthum* (ÖSTERGREN, l. c.) as in rye (MÜNTZING, 1946) the B chromosomes perform a non-disjunction at the first pollen mitosis and are included in the generative nucleus, the vegetative nucleus being without B chromosomes. As, in *A. aristatum*, the number of chromosomes is $2n = 10$ and plant No. 1 has 1 B chromosome, when the pollen grain gets 1 B chromosome the vegetative nucleus will get only 5 chromosomes, and the generative will get 7 (Figs. 9, 10, 14), but if the pollen grain does not get any B chromosome each nucleus will have 5



Figs. 1—18. Series in the course of division of the pollen nuclei from the normal case to the division of the vegetative nucleus without division of the generative. — Figs. 1—3, case 1, normal case. — Figs. 4—7, case 2, division of the vegetative nucleus after the division of the generative. — Fig. 8, case 3, probable division of the vegetative

chromosomes and they will be undistinguishable. It must be pointed out in this connection, however, that the utility of the B chromosomes in recognizing the vegetative and generative nucleus depends entirely on the truth of the assumption that the distribution mechanism of the B functions normally, an assumption which may not hold under these abnormal conditions.

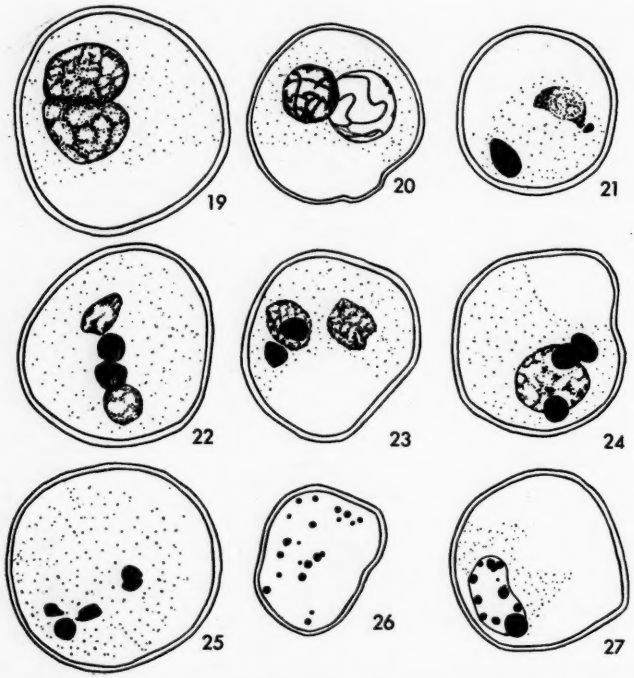
Through the synchronization of the division of the two nuclei they give origin to a common metaphase (Figs. 11, 12 and 13), which shows non-orientation of the chromosomes in the metaphase plate and sometimes strong contraction of the chromosomes (Fig. 13) like the prophase chromosomes of Figs. 9 and 10. What the destiny is of these two simultaneous mitoses could not be discovered with exactitude. We will come later to this point. A similar case of simultaneous but not syn-



nucleus when the generative is still at anaphase. — Figs. 9—13, case 4, synchronized division of the two nuclei and common metaphase. — Figs. 14 and 15, case 5, division of the generative nucleus after that of the vegetative. — Figs. 16—18, case 6, division of the vegetative nucleus without division of the generative. — × 1290.

chronized mitoses has been found by DARLINGTON and JANAKI-AMMAL (1945) in *Nicandra*, where the two nuclei divide at the same time but with a difference in phase. DARLINGTON and THOMAS (1941) have also found a common anaphase in *Sorghum* of two secondary central nuclei which have developed a third mitosis at their previous fusion, although this case differs from the *Anthoxanthum* case reported here, because in *Sorghum* the two nuclei are of vegetative nature while in *Anthoxanthum* one may be a generative and the other a vegetative nucleus, at any rate in many cases.

Case five shows (Fig. 14) the division of the generative nucleus (7 chromosomes) still at prophase when the vegetative is already at anaphase, in Fig. 15 the two vegetative nuclei were formed and the generative nucleus is reaching metaphase (note contraction of the chromosomes).



Figs. 19 and 20. Pollen grain with non-differentiated nuclei (Fig. 19) or weak differentiated nuclei (Fig. 20). — Fig. 21, pollen grain with chromatin drop. — Figs. 22—24, pollen grains with 4 nuclei considered as the result of supernumerary mitosis. — Figs. 25—27, pollen grains with a chromatin constitution considered, probably, as the result of supernumerary mitosis. — × 1290.

Case six shows the non-division of the generative nucleus accompanied by the division of the vegetative. The generative nucleus is, as usual, recognized by its smaller size and stronger staining ability. In Fig. 16 it is at prophase, in Fig. 17 at metaphase (note contraction and irregular distribution), and in Fig. 18 at anaphase.

These six cases show a complete seriation in the asynchronism of the division of generative and vegetative nuclei, which rises from the normal case of the division of the generative nucleus without division of the vegetative, passing through the complete synchronization of the division of the two nuclei to the division of the vegetative nucleus without any division of the generative.

The result of these supernumerary mitoses could not be definitely

ascertained. However, some observations that have been brought together in Figs. 22 to 27 show that in some cases the two divisions give origin to four nuclei and that of these four nuclei 2 are of a generative type and 2 of a vegetative type (Figs. 22 and 23), while in other cases 4 nuclei are formed which are not so well marked in type, for instance Fig. 24, which shows a pollen grain with 4 nuclei, 3 of which are of a generative type and one of a vegetative type, and also a case with 4 nuclei (2 fused) with a vegetative appearance, and another (Fig. 25) with 4 nuclei, two of which have not a regular nuclear shape; we consider them to be most probably the result of a common metaphase. In other cases it is possible to find pollen grains full of drops of a chromatin nature (Fig. 26) which most likely are another result of a common metaphase. In another case these chromatin drops appear in a prophase nucleus that seems to have degenerated before proceeding any further.

Chromatin drops. — Only in plant 1 has there been found a large quantity of pollen grains with chromatin drops in the cytoplasm (Fig. 21). In other plants only one or two pollen grains were found that contained them.

These drops appear in pollen grains with one, two and three nuclei but especially in uninucleate pollen grains, less often in binucleate ones and only a few in trinucleate pollen grains.

Contrary to the data obtained by MÜNTZING (l. c.) in rye, the frequency of chromatin drops in the present case is higher in uninucleate pollen grains than in binucleate ones. This shows that most probably these drops have their origin from the chromosomes of the normal complement and not from the B's, as most likely was the case in rye. Because, if the chromatin drops were from the B's, owing to the fact that the B's delay division (ÖSTERGREN, l. c.) those pollen grains which have chromatin drops will not get the B's in their nucleus and hence on an average they will divide more quickly than the others. But, as we can see from the data of Table 2, this is not what happens, the pollen grains that have chromatin drops are not hastened, but are on the contrary delayed in their division. Thus the chromatin drops most probably have their origin in the chromosomes of the normal complement. The elimination of a normal chromosome from the nucleus is most likely, either strongly to retard mitosis of that pollen grain or completely to inhibit it. No doubt, these drops indicate some asynapsis during meiosis in this plant. There is one more reason to show that

TABLE 2. *Relation between chromatin drops and division.*

Number of chromatin drops	Number of pollen grains with 1 nucleus	Number of pollen grains with 2 nuclei
0	24	20
1	82	13

they must derive from the normal complement; B's are not eliminated at meiosis, not even when unpaired (ÖSTERGREN, l. c.).

The analysis of these data indicates a significant difference, thus we are able to admit a positive correlation between chromatin drops and delay in division.

The presence of chromatin drops is not correlated to the presence of B's in another respect, that is to say, they appear only in plant 1 which has 1 B chromosome and not in other plants with the same number of B's or in plants with a higher number (2 or 4). It consequently seems that they most probably have their origin in an unbalanced genetic constitution of the plant, an unbalance to which the B most probably does not contribute at all or, mostly, to a minor extent.

DISCUSSION.

Relation between orientation and differentiation. — Non-differentiation is known to occur in a low frequency (7 %) under normal conditions in *Tradescantia gigantea* (SAX, 1937 b), and can be produced by different agencies. SAX (1935) has been able to produce the lack of orientation and differentiation in the pollen grains of *Tradescantia* by cold as well as by heat-treatment, and has found that the degree of differentiation of the two nuclei is closely associated with the angle of division. Temperature seems to affect cytoplasmic movement though not so much nuclear division. In a single anther SAX has found all degrees of differentiation, including the normal condition. He has also observed that heat-treatment is able to induce non-differentiation in the microspores of *Pseudolarix amabilis*, but in this case a difference was found from the *Tradescantia* case, that is, the differentiation of the two nuclei was not directly dependent upon the spindle orientation but upon the position more or less close to the wall of one of the two nuclei. In this case differentiation seems to be regional, that is to say, to be associated with some regions of the cytoplasm of the pollen grain (in this case the wall). Thus, it seems that differentiation is connected neither ab-

solutely with orientation nor absolutely with region, but it is connected with cell architecture, which in one case is expressed in one way and in another case in another way, and this architecture is the result of synchronization of the cytoplasmic and nuclear activities.

KOLLER (1943) could also obtain a loss of differentiation by means of the action of X-rays upon the pollen grains of *Tradescantia*, in which case the lack of differentiation was positively correlated to the presence of micronuclei.

An observation showing that differentiation is not directly connected with orientation is that made by SAX and HUSTED (1936) in *Periploca sepium*. The pollen grains of this species do not separate, remaining in tetrads, and the spindle of the microspore division may be in any plane, but »differentiation of generative and tube nuclei is complete, regardless of their orientation in the cell».

Thus we are able to conclude that orientation is not always directly connected with differentiation, which shows that simultaneity does not necessarily imply connection. The two phenomena seem to depend more on the timing of the activities of the nucleus and cytoplasm.

Relation between non-differentiation and supernumerary mitosis. — The relation between non-differentiation and supernumerary mitosis seems to be of the same kind.

There are already some cases in which supernumerary mitosis has been recognized (Table 3). In normal conditions it has been reported by SAX (1935) in a plant of *Tradescantia* growing in a greenhouse, and in normal plants of *Sorghum* by DARLINGTON and THOMAS (l. c.).

Supernumerary mitosis has also been induced under experimental conditions. In *Hyacinthus*, after heat-treatment, DE MOL (1923) found pollen grains with 2 to 8 globular, non-differentiated nuclei. By the action of temperature in *Tradescantia* SAX (1935) obtained supernumerary mitosis, accompanied by lack of differentiation. In *Nicandra*, which species contains a peculiar isochromosome, DARLINGTON and JANAKI-AMMAL (l. c.) also found a second division of the vegetative nucleus attended by non-differentiation (in 25 % of the pollen grains of an anther from a single plant).

In the material of *Anthoxanthum* discussed here one plant showed supernumerary mitosis of the vegetative nucleus accompanied by non-differentiation, while the other six plants all had non-differentiation without supernumerary mitosis. Non-differentiation seems to be connected, though not directly, with supernumerary mitosis.

TABLE 3. *Supernumerary mitosis in microspores.*[1]

Normal conditions	Experimental conditions	In plants with unbalanced genetic constitution
Tradescantia, SAX (1935) *Sorghum*, DARLINGTON and THOMAS (1941)	*Hyacinthus*, DE MOL (1923) *Tradescantia*, SAX (1935)	*Tradescantia* (triploid), SAX (1937 b) *Amaryllis* (polyploid), UPCOTT (1939) *Sorghum* (B's), DARLINGTON and THOMAS (1941) *Nicandra* (isochromosomes), DARLINGTON and JANAKI-AMMAL (1945) *Anthoxanthum* (B's), the present paper

[1] The cases recorded by BEADLE (1931) and JOHNSSON (1944) are considered as supernumerary mitosis somewhat different from those listed here.

Relation between supernumerary mitosis and unbalanced genetic constitution. — DARLINGTON and THOMAS (l. c.) found in their material a relation between supernumerary mitosis and B chromosomes. In *Sorghum*, where they found supernumerary divisions of the vegetative nucleus, they observed that the extra divisions are stimulated by the presence of B's, having noted in all the 20 pollen grains studied in this respect that B chromosomes were present at the first extra pollen division. Anyhow, this is not exactly the case in *Anthoxanthum*, as can be seen from the presence of B's in the supernumerary mitosis of Figs. 9, 10 and 14 and their absence in Fig. 15.

These authors have also observed a variation in intensity of supernumerary divisions, conforming with a variation in the frequency of B's. By a glance at Table 1 it is possible to see that this is not the case in *Anthoxanthum*: the plants with the highest number of B's have no supernumerary mitosis, and of plants with the same number: one has and the others have not.

DARLINGTON and JANAKI-AMMAL (l. c.) found a second division of the vegetative nucleus in *Nicandra* with isochromosomes. In polyploid material as *Amaryllis Belladonna* (UPCOTT, 1939) and triploid *Tradescantia* (SAX, 1937 b) a second division of the vegetative nucleus has also been observed several times in *Amaryllis* and occasionally in *Tradescantia*.

This indicates that supernumerary mitosis may sometimes be correlated to a genetic unbalanced constitution, but we can see from the facts here reported from *Anthoxanthum* that the correlation is not absolute.

Conclusions. — If life is considered to be dependent upon a series of reactions which normally proceed at rates bearing a definite relation to one another, an idea pointed out by OSTERHOUT (1921), it is clear that a disturbance of these rate relations should have profound effects upon the organism.

Disturbances in such rate relations are known to have different origin and to lead sometimes to the same phenotypical result, as is known from GOLDSCHMIDT's works (GOLDSCHMIDT, 1938); and, as SAX (1937 a) reported, abnormalities induced by temperature treatment are similar to such induced by narcotics, osmotic pressure, X-rays, genetic factors, and other agencies. Most of these various agencies seem to induce abnormalities in cell and nuclear division by producing changes in the synchronization of nuclear and cytoplasmic activities.

The abnormalities reported in this work, viz. lack of orientation, non-differentiation, asynapsis (chromatin drops) and supernumerary mitosis, seem to be of this kind. They can have their origin in environmental conditions or in the unbalanced genetic constitution of the material. Thus they are most probably connected, though only because they are different expressions of the same cause — disturbances in timing.

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SUMMARY.

In the pollen grains of *Anthoxanthum aristatum* BOISS. with B chromosomes, lack of orientation and non-differentiation of the nuclei have been found in 7 plants with from 1 to 4 B's.

A complete seriation between the division of the generative nucleus unaccompanied by that of the vegetative nucleus (the normal case) and a division of the vegetative nucleus unaccompanied by that of the generative has been observed. Synchronized mitoses of both generative and vegetative nuclei, giving rise to common metaphase, are presented.

A positive correlation between chromatin drops and delay in division is recognized. Their origin is attributed to chromosomes of the normal complement.

A discussion of the relation between these phenomena is developed; the conclusion is drawn that, most probably, they are different expressions of disturbances in timing relations.

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CONTENTS.

Page
Non-orientation, weak or non-differentiation of the two nuclei 539
Supernumerary mitosis, chromosome contraction and spindle disturbances 540
Chromatin drops 545
Discussion 546
Relation between orientation and differentiation 546
Relation between non-differentiation and supernumerary mitosis 547
Relation between supernumerary mitosis and unbalanced genetic constitution 548
Conclusions 549
Acknowledgments 549
Summary 549
Literature cited 550

THE RESISTANCE OF COLCHICUM AND BULBOCODIUM TO THE C-MITOTIC ACTION OF COLCHICINE

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I. INTRODUCTION.

SINCE *Colchicum autumnale* in its tissues contains a considerable amount of colchicine — up to 1 % —, its own mitoses would be expected to be unaffected by this substance. In most higher plants the mitotic mechanism is switched over into so-called c-mitosis under influence of colchicine concentrations very much lower than those present in *Colchicum*. During c-mitosis any increase in cellular number is made impossible. Different authors have shown that pulp from crushed tissues of *Colchicum* may induce full c-mitosis in other plants. BLAKESLEE (1939) was the first one to point out the resistance of *Colchicum* to colchicine. He found that the c-tumour reaction could not be obtained in *Colchicum* by colchicine treatment. The behaviour of the root mitosis in *Colchicum* during long treatment with 1 % colchicine solution was studied by LEVAN (1940), who found no tendencies to c-mitosis. In 1943 MAIROLD repeated the testing of *Colchicum* with concentrations of crystalline colchicine up to 1 % in temperatures reaching + 30 centigrades. (In the present paper we always understand by »crystalline colchicine» the chloroform-containing crystalline form of the alkaloid.) MAIROLD found an absolute resistance as far as the c-tumour reaction was concerned: »So wurde auf diese Weise erneut die weitgehende Immunität der *Colchicum*-Wurzeln gegenüber Colchicin bewiesen, selbst unter Bedingungen, bei denen das Colchicin sonst besonders intensiv wirkt» (l. c., p. 494).

An American author, CORNMAN (1941, 1942), has re-tested the reaction of *Colchicum* to colchicine and arrives at another result than the above workers. He employed a very delicate method: the upper ends of free *Colchicum* roots were embedded in agar with 5 % glucose, while the lower ends were dipped into the solutions to be tested. Mitoses could still be found in the severed roots after a treatment of 8 $^1/_2$ hours. According to CORNMAN, 5 % colchicine for 4 hours give complete spindle

destruction in *Colchicum byzantinum*, the corresponding values for *Colchicum autumnale* were a 10 % solution for 6 hours. The latter species was thus more resistant than the former one, the resistance of neither of them being absolute.

As a continuation of our work on the action of c-mitotic substances we decided to re-investigate the resistance of *Colchicum*. Among the above authors only MAIROLD gave particulars as to what kind of colchicine she used, viz. MERCK's colchicine cryst. with 12,5 % chloroform. It may be communicated here that LEVAN (1940) in his testing of *Colchicum* used SANDOZ' colchicine amorph. Since it has been shown that pure chloroform may induce typical c-mitosis in *Allium* (ÖSTERGREN, 1944; STEINEGGER and LEVAN, 1947 b), it was close at hand to direct attention to the chloroform content of the crystalline colchicine. In the earlier paper just mentioned we studied the influence of the chloroform contained in crystalline colchicine ($C_{22}H_{25}NO_6 \cdot {}^1/_2CHCl_3$) on a plant not resistant to colchicine, viz. *Allium Cepa*. We arrived at the conclusion that the content of chloroform present in this colchicine is of no consequence in the low concentrations at which the threshold value for c-mitosis is situated in *Allium*, i. e. about 0,004 % colchicine. In the present work two plant species resistant to colchicine are studied. At such strong concentrations of colchicine as have been tested in *Colchicum*, crystalline colchicine would, however, hold a chloroform content which might well be expected to give c-mitosis in this plant, provided it is not resistant to chloroform.

MAIROLD, using 1 % of crystalline colchicine, obtained a negative result as regards the c-tumour reaction. This fact, however, cannot be considered a conclusive evidence that *Colchicum* is absolutely resistant, since c-tumours are hard to induce by chloroform owing to the toxicity of this substance. The mitoses were not studied by MAIROLD. In the case of CORNMAN it is not recorded what quality of colchicine was used. The easy preparation of 10 % solutions would indicate that a chloroform-free substance was used. We have found, however, that the chloroform-containing substance may also be rather easily dissolved to 10 %, if the fluid is shaken or heated. The chloroform will then evaporate in part. Even in the clear solution undoubtedly enough of the chloroform may remain to give c-mitosis.

In order to elucidate this problem a little the present work was started. We tested *Colchicum autumnale* for its reaction to colchicine puriss. amorph. SANDOZ (without chloroform), to pure chloroform, to colchicine puriss. cryst. DAB 6 MERCK (with 12,5 % chloroform) and to

acenaphthene. Besides *Colchicum* we tested in a similar way the related species *Bulbocodium vernum*, belonging to the same section. Both experimental plants were bought from Weibullsholm, Landskrona.

The same simple method was employed in this work as in our earlier experiments. Bulbs with fresh root-tips in active growth were put into series of concentrations of the various substances. Only in a few cases (the testing of *Bulbocodium* with chloroform and some of the testings of *Colchicum* with chloroform) have we used another mode of procedure, this being made necessary by lack of plant material. Detached root-tips were submersed for 4 hours in solutions of the substances. Tests in *Allium* have shown that free roots thus treated during 6 hours give results fairly concordant with those obtained for attached roots.

The chromosomes of *Colchicum autumnale* have been studied by LEVAN (1940). The chromosome number is $2n = 38$. Great variations in chromosome size occur within the idiogram. Fig. 1 *a* shows a normal equatorial plate from an acenaphthene treatment. The chromosomes are somewhat contracted, which facilitates the counting. *Bulbocodium vernum* has been earlier studied by MILLER (1930), who gives its chromosome number as $2n = 22$. One plate from a 4 hours' treatment with 0,4 % colchicine cryst. is pictured in Fig. 1 *b*. Also here the chromosome contraction is somewhat stronger than normally. The plate is in this respect quite comparable to Fig. 1 *a*. The chromosomes of *Bulbocodium* are somewhat larger than those of *Colchicum*. Most of them are medially to submedially attached, but at least one of the smallest pairs is asymmetric with one arm and one small head. The size differences are less extreme than in *Colchicum*. The largest chromosomes of *Bulbocodium* are about 5 μ and of about double the length of the smallest ones. Although the idiograms of *Colchicum* and *Bulbocodium* are of the same general type, it is quite clear that no simple relation of polyploidy can exist between the two types. The largest chromosomes of *Colchicum* and *Bulbocodium* agree pretty well morphologically, but the smallest chromosome types of *Colchicum* are not present in *Bulbocodium*.

II. EXPERIMENTS.

a. *Amorphous colchicine.* — This form is, compared with crystalline colchicine, highly soluble in water. Strong concentrations are, however, not stable, since after even a short time they form crystals of water-containing colchicine. This latter substance is soluble in water only to about 1,4 %. The strongest concentration of amorphous colchicine

tested by us is 20 % (0,5 mol; % = g substance in 100 cc solution). This solution kept clear for about 30 min., when it started crystallizing. On the first fixing (after 4 hours) the amount of crystals was estimated to be certainly below one-fourth of the dissolved quantity; so at that time the solution was still at least 15 %. Solutions down to 5 % were tested in *Colchicum* and down to 0,4 % in *Bulbocodium*. The toxic effect of pure colchicine is very insignificant in the two plants. A treatment of *Colchicum* for 11 days with an originally 20 % solution impeded, it is true, the further growth of the roots. Mitoses occurred, however, in the meristem, and they were quite normal. A fixation of *Bulbocodium* grown 4 days in the same concentration showed no perceptible toxic action. Although the root-tips were quite covered with colchicine crystals, they kept turgescent. In all lower concentrations mitoses were going on normally during the whole period of the experiment. No c-tumours were found.

We looked carefully for c-mitoses and signs of spindle destruction without any positive result. The mitoses and spindles were perfectly normal in all fixations. In the highest concentration a pronounced stickiness was met with, especially at the first fixing (after 4 hours); at the second fixing (after 24 hours) the stickiness was decidedly less marked. Fig. 1 *c—f* shows instances of mitoses from this treatment. As seen, the stickiness could be so severe as to cause lumping of the chromosomes, sometimes simulating certain c-mitotic features. The spindles were always present, however. In rare cases tendencies to small spindle disturbances were seen, as very wide poles with traces of multipolarity (Fig. 1 *c—d*). All anaphases studied were bipolar, however. Fig. 1 *f* shows all chromosomes gathered in the middle of a cell. It is strongly reminiscent of ball-metaphase but may possibly be an early metaphase stage, where the spindle has not yet acted on the chromosomes. This picture, which is very common in *Colchicum* roots showing c-mitosis, was very rare here. It must be concluded that no typical c-mitotic disturbances are induced by pure colchicine. The abnormalities seen in the highest concentrations may be ascribed to agglutination phenomena.

b. *Iso-colchicine.* — In *Colchicum* one experiment was made with *iso*-colchicine (concerning the effect on *Allium* of this substance, cf. STEINEGGER and LEVAN, 1947 a). Only one concentration was tested, viz. 1 % (0,025 mol). This rather strong solution (it is about half-saturated) gave no c-mitosis. It brought about a somewhat more pronounced stickiness than ordinary colchicine. The metaphases often

turned pycnotic and the anaphase chromosomes formed bridges (Fig. 1 *g*). The spindles were always normal. The substance had no great toxic effect, after 6 days the roots were still turgescent. They showed little further growth. No c-tumours were observed.

c. *Chloroform.* — Chloroform which accompanies colchicine in its crystalline form was tested in both plants in a series of the following concentrations: 0,5, 0,25, 0,10, 0,05, 0,025 % by weight. The activity threshold of *Allium* was known to lie at about 0,025 % (STEINEGGER and LEVAN, 1947 b). In *Allium* we found the strongest concentration to have a peculiar effect, it acted dissolving on the chromosomes, in extreme cases resulting in the complete disappearance of the chromosome arms, leaving the centromeres quite free. In *Colchicum* it turned out that one hour's treatment with 0,5 % chloroform gave the same effect. In *Bulbocodium* we fixed the material only once, viz. after 4 hours. Then the dissolution of the chromosomes had gone too far. The chromosomes at contraction stages had completely disappeared, the cells being quite empty. In the nuclei only the largest heterochromatic bodies were still to be seen. They formed 1 to 5 strongly stained bodies.

In *Colchicum* the various stages of the chromosome cycle were clearly seen. No c-mitotic changes occurred, this concentration of chloroform probably killing the cells before they were able to give any vital chromosome reactions. The chromosomes were at various stages of dissolution. Fig. 1 *h—j* shows one metaphase and two beginning anaphases. In *h* much of the chromatic substance is still gathered around the stained centromeres. In *i* the half-centromeres probably have not yet separated, in each chromosome they form two distinctly stained grains. They are smaller than the corresponding formations in *Allium* and they seem to be less compound morphologically, but, of course, with these small dimensions nothing can be said as to their detailed organization. In Fig. 1 *j* the centromeric points of each pair are sometimes rather far away from each other; they are evidently on their way to the poles. In this picture every trace of the chromosome arms had vanished and the centromeres themselves were faintly stained. It will seem that the dissolution proceeds in the same way as in *Allium*, taking away first the matrix substance, later the chromonema, and last the centromeres.

The next concentration, 0,25 %, is also lethal. The *Colchicum* roots were turgescent for a couple of hours in this concentration but after 4 hours they were already somewhat softened. This concentration also has a fixing action, but in *Colchicum* tendencies to c-mitoses were dis-

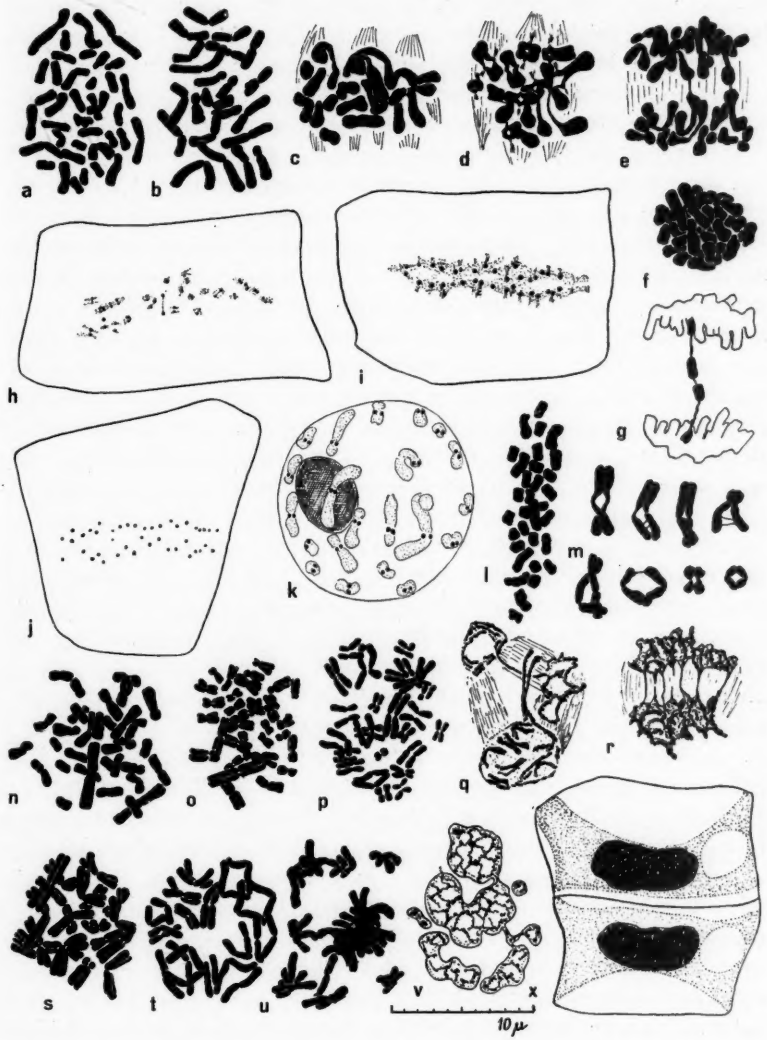


Fig. 1. *a, c—r*: *Colchicum autumnale*, *b, s—x*: *Bulbocodium vernum*; *a—b*: normal equatorial plates with chromosomes somewhat contracted from a slight c-mitotic treatment; *c—f*: treatment with amorphous colchicine; *g*: iso-colchicine; *h—j*: chloroform; *l*: crystalline colchicine; *k*, *m—x*: acenaphthene. — × 2200.

cernible, since the chromosomes have had time to respond to the spindle destruction. Toxicity was very strong, all division stages being pycnotic.

In 0,1 % both *Colchicum* and *Bulbocodium* exhibited 100 % c-mitosis. Chromosome contraction was strong. Stickiness occurred but not to any high extent. The chromosomes were mostly lying free from each other, forming typical c-pairs. The concentration 0,05 % also gave full c-mitosis in *Colchicum* on testing detached roots. On testing roots still on the bulbs this concentration gave preponderantly normal mitoses. In *Bulbocodium*, where this series was made exclusively with detached roots, only few mitoses were present in this concentration, among them being both normal and c-mitoses. It is clear that both in *Colchicum* and *Bulbocodium* the activity threshold is approached: 0,025 % gives in *Colchicum* (detached roots) mixed c- and normal mitoses. *Bulbocodium* had no mitoses in this concentration. Thus, even if the lower limit of c-mitosis could not be exactly determined by these experiments, it is evident that it must be situated in the vicinity of 0,025 %, i. e. it is of the same size-order as in *Allium*.

Chloroform had a very pronounced toxic effect throughout all concentrations. In the lower concentrations it was especially clear after 24 hours, when no mitoses at all occurred. The highest concentration, 0,5 %, gave slack roots in less than 45 min. As mentioned already, the roots in 0,25 % began softening after 4 hours. After 16 hours they were quite soft. The 0,1 % solution also gave some defective turgescence after 5 days. None of the concentrations induced c-tumours, not even 0,05 %, which in *Allium* gave clear tumours.

d. *Crystalline colchicine.* — Solutions between 4 % and 0,5 % of chloroform-containing colchicine were tested in *Colchicum*, and between 4 and 0,01 % in *Bulbocodium*. These solutions are calculated to contain 12,5 % chloroform, which corresponds to chloroform concentrations of 0,5—0,06 % in *Colchicum* and 0,5—0,001 % in *Bulbocodium*. The toxicity of colchicine cryst. is much greater than of the same concentrations of colchicine amorph. It is somewhat less than that of corresponding concentrations of pure chloroform. The latter condition may be due to the calculated concentrations of chloroform actually being lower. Some chloroform may have evaporated at the preparation of the colchicine solutions. Moreover, the tests with colchicine were made with less fluid in lower vessels of larger surface in relation to the volume of fluid than when pure chloroform was tested, which may have brought about a relatively greater evaporation in the colchicine series. Thus, the smell of chloroform, which was very evident at first, had usually disappeared

totally after one day. The corresponding solutions of pure chloroform still smelt of chloroform after this time. The possibility that in some way or other the colchicine may abate the toxic action of chloroform cannot yet be rejected, however.

In the strongest concentration, 4 %, there should be present 0,5 % chloroform. This solution, however, has quite other effects than a pure chloroform solution of 0,5 %. It has not a fixing action. The roots of *Colchicum* were still turgescent on the third day, in *Bulbocodium* they softened on the second day. Chromosomal stickiness occurred, it is true, but not by far such an extreme dissolution of the chromosomes as in 0,5 % chloroform. In both species 100 % c-mitosis was found after 4 hours and the chromosome contraction was strong. Fig. 1 *l* shows a c-metaphase from this treatment. As early as after 24 hours, however, most mitoses had become normal, c-mitosis occurring only exceptionally. In *Bulbocodium* no divisions were found after 24 hours, the toxic action was such that divisions were very seldom found on the second occasion of fixation.

Solutions of 2 % and 1 % crystalline colchicine also gave 100 % c-mitosis in both species. In the latter concentration, though, other types of c-mitoses began to appear, viz. »star» and »distributive» metaphases, which indicates that the limit value is not far away. After 24 hours *Colchicum* showed preponderantly normal mitoses in 2 % and almost exclusively normal mitoses in 1 %. As mentioned above, *Bulbocodium* had no mitoses on the second day. The 0,5 % concentration had exclusively normal mitoses in *Colchicum*, as had 0,4 % and all lower ones in *Bulbocodium*.

C-tumours were demonstrated in *Colchicum* only in one concentration, viz. 0,5 % after four days. This agrees with the threshold of chloroform in *Allium*, where 0,05 % and 0,025 %, but not 0,1 %, induced c-tumours. A 0,5 % crystalline colchicine concentration contains 0,06 % chloroform, or probably somewhat less.

e. *Acenaphthene.* — This substance has earlier been tried on *Colchicum* only in qualitative tests. LEVAN (1940) dusted the roots with acenaphthene and let them thereafter grow in moist air. Under these conditions *Colchicum* was very prone to acquire typical c-tumours and c-mitoses. CORNMAN (1942) reports complete resistance to acenaphthene: »Mitosis continued normal in *C. autumnale* during 7 hours exposure to saturated aqueous acenaphthene» (l. c., p. 55).

The solubility of acenaphthene in water is about 20×10^{-6} mol/l (LEVAN and ÖSTERGREN, 1943). In a similar way as described in that

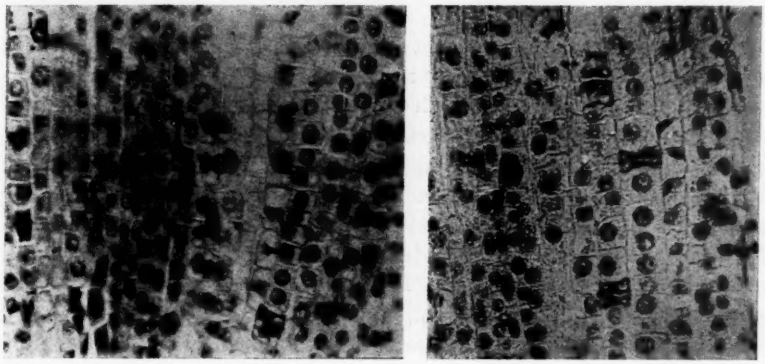
paper, solutions of acenaphthene were prepared, containing a varying excess of undissolved substance. The strongest concentration corresponded to a molarity of 100×10^{-6}. In *Colchicum* concentrations down to 25×10^{-6} mol/l were tested, in *Bulbocodium* we went down to 1×10^{-6} mol/l. In no concentration did 100 % c-mitoses occur. In *Colchicum* an exposure to 100×10^{-6} mol/l gave in 4 hours numerous complete c-mitoses with strong chromosome contraction. Various instances of c-mitoses from this treatment are pictured in Fig. 1 *n*—*r*. An often occurring stickiness is apparent both in the contraction stages of the chromosomes and, above all, in the bridge formation at anaphase—telophase (Fig. 1 *q*—*r*). There was a similar response in *Bulbocodium* to the same treatment. C-mitoses which end with multipolar anaphases and lobated telophase nuclei are common (Fig. 1 *s*—*v*).

Colchicum has a great frequency of c-mitoses in 50×10^{-6} mol/l as well. Stickiness is still evident, for instance, at the separation of the c-pairs at anaphase (Fig. 1 *m*). The centromeric region separates readily but the chromosome arms are glued together, strings of sticky chromatin are often formed between the chromosome arms on their falling apart. In *Bulbocodium* this concentration has a low frequency of c-mitoses, only single tendencies being seen. The 25×10^{-6} mol/l concentration in four hours shows in *Colchicum* still a few certain c-mitoses, in *Bulbocodium*, on the other hand, exclusively normal mitoses are found. The latter seems to be somewhat less sensitive to the c-mitosis reaction of acenaphthene than the former. Unfortunately no lower concentrations of *Colchicum* were studied, but it may be stated with great probability that the threshold of *Colchicum* is situated immediately below saturated solution of acenaphthene, while in *Bulbocodium* a certain excess of undissolved substance is necessary to attain the reaction.

One very interesting condition is characteristic of both *Colchicum* and *Bulbocodium*: the toxicity of acenaphthene, which after 4 hours is very small, is already quite pronounced after 24 hours. This is the case not only with the higher concentrations but goes down to the lowest ones tested (in *Bulbocodium* to 1×10^{-6} mol/l). In the entire *Bulbocodium* series hardly any normal division stages could be found on the second occasion of fixing. This was already seen macroscopically in that after a few days the roots grew brownish and ceased elongating even in the weakest solutions. As mentioned already, conditions were similar in the chloroform concentrations and to a certain degree in the colchicine cryst. series. In acenaphthene the roots kept turgescent and it happened that later on, after 4 days, new divisions began to appear,

although never in any great frequency. After 3 days clear c-tumours were started in *Colchicum* in 100 and 50×10^{-6} mol/l and tendencies to c-tumours in 25×10^{-6} mol/l. In *Bulbocodium* only very weak c-tumours were induced in 100 and 50×10^{-6} mol/l. It is evident, however, that cellular growth is not inhibited by the type of toxicity acting here.

Even in very low concentrations acenaphthene exerts a specific kind of toxicity on the meristems, a toxicity which is not known from the acenaphthene treatments of *Allium*. It takes an interesting expres-



a *b*

Fig. 2. Microphotographs of root meristems of *a*: *Colchicum*, *b*: *Bulbocodium* after treatment for 24 hours with acenaphthene. Single cells or pairs of cells are dead. — $\times$ 250.

sion in the histology of the meristems. Certain cells die in the tissue. These cells are always rather isolated and, at least to begin with, the neighbouring cells are not affected. The meristem becomes scattered with solitary or paired dead cells. On a closer study of these cells it is found that they all have the common trait that they were in division stages of mitosis when dying, in metaphase or anaphase. Here evidently a selective cell lethality is in action, choosing cells in division. The threshold of this lethality lies far below the c-mitosis threshold. This toxicity is strongly specialized, distinguished from the general toxicity which impedes cellular growth. One instance of a couple of anaphase or telophase cells killed by this toxicity is pictured in Fig. 1 *w*. A tissue section of *Bulbocodium* and *Colchicum* affected by this type of toxicity is photographed in Fig. 2.

In the strongest concentration, 100×10^{-6} mol/l, there occurred in *Colchicum* after 4 days a very distinct centromeric staining during prophase. Fig. 1 *k* shows the lower half of one such nucleus with the chromosomes scattered on the nuclear membrane. All had their centromeres showing a distinct colour, while the rest of the chromosomes was faintly stained. As seen, the centromeres were double in transversal direction. They had about the same size and appearance as the »free» centromeres occurring after 0,5 % chloroform treatment.

III. DISCUSSION.

The present experiments have given conclusive evidence that both *Colchicum* and *Bulbocodium* are resistant to colchicine. Their resistance keeps in concentrations as high as 20 %. The mitotic disturbances occurring in the highest concentrations of amorphous colchicine are not c-mitotic, but they are caused by the agglutination phenomena, which are often called stickiness. These may be induced by a great many chemical substances, among them many c-mitotic substances. *Colchicum* is also found to be resistant to *iso*-colchicine, even in concentrations which in *Allium* give c-mitosis. *Bulbocodium* was not tested with this substance.

The two plant species are also resistant to chloroform-containing colchicine up to concentrations in which the chloroform content reaches above the threshold value of c-mitosis. Then complete c-mitosis is obtained with crystalline colchicine. The results of CORNMAN (l. c.) may be interpreted as depending on his using a chloroform-containing substance. With the guidance of our experiments it is even possible to calculate approximately the content of chloroform which the substance used by CORNMAN must have held: 5 and 10 % in some cases gave complete c-mitosis, 2,5 % only gave partial. Consequently, at the treatments the colchicine substance should have contained 1 to 2 % chloroform.

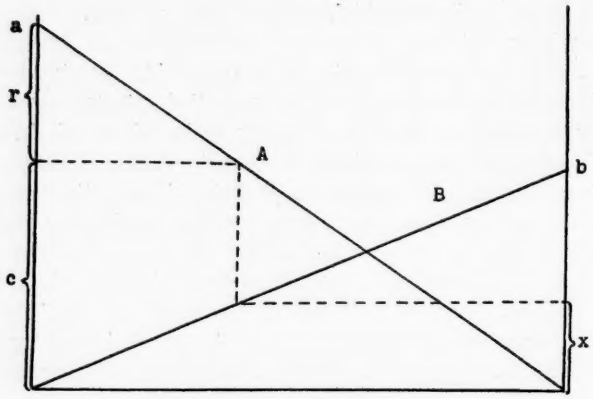
Even the strongest solutions tested of pure colchicine hardly act poisonously, while chloroform far down in the dilutions brings about considerable poison effects. Crystalline colchicine also acts toxically, although to a less degree than corresponding concentrations of pure chloroform. In *Bulbocodium* 4 and 2 % crystalline colchicine acted very poisonously, and a toxicity consisting in the inhibiting of all mitoses acted in colchicine concentrations of 0,01 %, i. e. a chloroform concentration of 0,001 %. What was especially surprising was the pronounced

toxicity of acenaphthene, which when used on other materials has shown very little toxicity.

The entire problem of resistance gains considerably in clearness if the c-mitotically active substances are classified into two groups, such with mainly unspecific, physical activity and such which in addition have a chemical activity, varying from substance to substance. While the substances of the former group probably induce the c-mitosis by some similar quality common to them all, the specifically acting substances of the latter group may attain their effect by attacks on any one of a number of different life processes of the cell; it may be a question of either a direct chemical influence on the components of the spindle or such an influence on ferment systems directly or indirectly involved in their synthesis. Only in their visible final effect do they agree in all cases: the morphological deviation of mitosis, called c-mitosis.

In a similar way it will be appropriate to divide the phenomena of resistance into two groups, unspecific, physical on one hand and specific, chemical on the other. A physical resistance (=decreased susceptibility), which, of course, can only be relative, may be due, e. g., to some deviating physical properties of the spindle constituents. The physical resistance may be extended to the whole group of unspecific (»narcotic«) poisons. This situation may be illustrated by the simple Scheme 1, constructed in accordance with ÖSTERGREN's schemes (1944, Fig. 11, p. 458). According to the hypothesis of ÖSTERGREN, the c-mitotic action is caused by an influence on the lipophilic side-chains of the polypeptid molecules. At a certain degree of »saturation« the chains are folded and the spindle formation is made impossible. ÖSTERGREN compares this process with the precipitation of a dissolved substance by adding another substance to the system. *a* and *b* of Scheme 1 represent molar concentrations of a saturated solution of the substances *a* and *b*. If the concentration of *a* sinks along the line *A*, it will be necessary, in order to obtain saturation of the solution, to add such a molar concentration of substance *b* as is expressed by the line *B*.

If this is adapted to the conditions of the c-mitosis, *a* will mean the »concentration« of lipophilic side-chains and *b* the concentration of a c-mitotic substance. Now, if *c* means the degree of »saturation« of the protein chains as regards lipophilic side-chains, the quantity x of the substance *b* necessary for inducing c-mitosis may be obtained by following the dotted line. From this it appears that the values of x increase simultaneously as *c* decreases. In other words: the farther the concentration of lipophilic side-chains is from its »saturation« (expressed by



Scheme 1. (See text.)

the distance *r*), the more substance *b* must be added in order to obtain c-mitosis, the greater is consequently the resistance of the cell. This suggestion, naturally, is not necessarily linked to the hypothesis of »protein chain folding», but it will work equally well on any hypothesis assuming such a precipitation of lipophilic groups or molecules.

This situation is, of course, valid only with physically acting substances. The chemical resistance, on the other hand, must vary according to the point of attack of each particular substance. In each single case a certain resistance must be mobilized, directed to the specific attack in question. Thus, immunity to one chemically acting substance does not mean immunity to all substances of this group.

The following table may serve as an illustration of these conditions. As regards the chemical activity of $CHCl_3$, see our earlier paper (1947 b).

Group	Substance	Threshold concentration in %		
		Allium	*Colchicum*	*Bulbocodium*
more or less chemically acting	colchicine ...	0,004	>20	>20
	chloroform...	0,025	0,025	0,025
preponderantly physically acting	*iso*-colchicine	0,6	>1	?
	acenaphthene	0,000.08	0,000.4	0,000.8

As representatives of specifically acting substances colchicine and chloroform are given. The difference in behaviour between *Allium*, on

one hand, and *Colchicum* and *Bulbocodium*, on the other, is striking. While the latter species are completely resistant to the chemical influence of colchicine, they are as sensitive as *Allium* to the influence of chloroform. Their chemical resistance is accordingly specific. Further, two instances of preponderantly physically acting substances are given: *iso*-colchicine and acenaphthene. *Colchicum* is somewhat more resistant than *Allium* to acenaphthene, and the same is the case with *iso*-colchicine, which substance has, it is true, quite another chemical nature than acenaphthene but nevertheless acts preponderantly unspecifically. Although the values so far at hand are insufficient to permit any proof of the present view of the resistance problem, they must be considered as rather suggestive. LEVAN and ÖSTERGREN's results in *Allium* and *Pisum* (1943) may also be mentioned:

	Threshold value in 10^{-6} mol/l		
	Colchicine	Naphthalene	Acenaphthene
Allium	150	78	5
Pisum	75	156	32

These values also are not inconsistent with our view that chemical and physical resistance are independent of each other. While *Pisum* is more sensitive to chemical influence than *Allium*, its resistance to naphthalene and acenaphthene is greater. It seems as if an increased resistance to acenaphthene may also involve a similar increase in resistance to naphthalene. The unspecificity of the resistance to physical action is also present in the »camphor reaction» of yeast (LEVAN, 1947). An induced resistance to certain substances also brings about an increased resistance to other chemically quite unrelated substances.

SUMMARY.

The reaction of *Colchicum autumnale* and *Bulbocodium vernum* to amorphous (pure) and crystalline (chloroform-containing) colchicine, *iso*-colchicine, chloroform and acenaphthene is studied. Both species show an absolute resistance to the c-mitotic action of colchicine and *iso*-colchicine in high concentrations (20 and 1 % respectively). To chloroform their response is similar to other colchicine sensitive plants as, for instance, *Allium Cepa*. They both give typical c-mitoses if treated with such concentrations of crystalline colchicine as have a chloroform content above the threshold concentrations of this substance alone. Both

are susceptible to the c-mitotic action of acenaphthene. As explanation of the data on the susceptibility of *Colchicum* to colchicine encountered in the literature it is suggested that a substance containing chloroform may have been used. In agreement with the two types of action on the living cell of c-mitotic substances, viz. physical and chemical action, it is suggested that two types of resistance may occur: a physical, less selective resistance and a chemical specific resistance, which in each case is directed against a certain kind of attack. While in *Colchicum* and *Bulbocodium* the resistance to the chemical activity of colchicine is absolute, they are both as susceptible as *Allium* to another very probably chemically acting substance, chloroform. Both species are more resistant than *Allium* to acenaphthene and *iso*-colchicine (only *Colchicum* studied), which are mainly physically acting substances.

Svalöf, March, 1947.

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Communications from the Swedish Seed Association, No. 4.

ABSTRACTS — KURZE MITTEILUNGEN

GÖSTA VON ROSEN: The rapid nigrosine-method for chromosome counts, applicable to all the growing tissues of the plant.

A plant-breeder who works with cross-fertilizing kinds of plants and who desires to work up polyploid material on a large scale will soon discover that an annual control of the purity of the polyploid stocks as regards chromosome number is necessary. For even if he isolates the polyploid materials well by distance from other propagations, there will be so much pollen flying about in the air (e. g., in *Beta*) that some risk of incrossing with undesired chromosome numbers will constantly be present. A breeder of fruit and forest trees should also have at his command methods by which the chromosome numbers in new artificial or spontaneous crosses can be simply separated.

A grading of the polyploid chromosome-number groups only demands an estimation of the number of chromosomes in the genome (e. g., in *Beta* 36 ± 2—4). If, in addition, the method can often give metaphase plates so well pressed out that an exact count is possible, this is obviously an advantage. First and foremost, however, it must provide a rapid means of getting the preparations ready and of reading them (= well contracted chromosome bodies). The usual paraffin-gentian method as well as the Feulgen-smear method (e. g., JOHNSON, 1945; MEYER, 1943; WHITAKER, 1939) are too time-absorbing to be suitable for serial work. There are numerous variations of smear preparation with carmine or orcein as stain (e. g., BROWN, 1937; BURREL, 1939; VON ROSEN, 1946 a, b; SAX, 1931; SMITH, 1947; STEERE, 1931; THOMAS 1940; WARMKE, 1935; WHITAKER, 1934). All suffer, however, from the weakness that they only stain the chromatin red and in addition give a poor differentiation between chromatin and plasm. Heating is also included in the process of preparation, which makes the work delicate. Further, chromosome counting ought to admit of being done during the whole vital cycle of the plants, this enabling a failure at the beginning to be remedied later on. In *Beta*, for instance, now at Hilleshög the rootlets are forced artificially during the winter, and an after-control can be carried out later on leaves from the stem-tips from the commencement of growth up to the flowering. The pollen mother-cells can also be rapidly judged. *All this ought to admit of being done by the same simple method.* It would mean that dear, specially trained staff would not be required for the purpose, as a small number of persons engaged in other work during the rest of the year have good time at their disposal to carry out the program.

A rapid method for the plant-breeder for the grading of polyploid material should be of the following nature: (1) simple, (2) distinctly contrast-staining, (3) rapid, (4) adapted to serial work, (5) applicable to all the growing tissues of the plant, (6) cheap.

The method described below seems to meet these requirements. The treatment is essentially the same for roots as for leaves and flower-buds. Any deviations between them are noted in their proper connexion.

(1) *Fixing.* — Fix the material in 1 part of concentrated (98 %) acetic

acid + 2 parts of 95 % alcohol for about 24 hours. The preparation should be preserved in a cool state (not above 15° C., preferably 8—12° C.). The fixing solution must be mixed within 24 hours of being used.

(2) *Loosening of the tissues.* — Replace the fixing fluid with a mixture of 1 part of concentrated HCl (sp. gr. 1,27) + 2 parts of 95 % alcohol. Roots are treated for 8—10 minutes at a temperature of 10—15° C. (an important condition is that the fluid is cooled). For leaves the character of the tissues will decide the concentration and time: *Beta* 1 : 2 for 7—8 minutes, *Pisum* 1 : 4 for 5 minutes, *Pyrus* and *Betula* 1 : 1 for 10 minutes, and so on. Flower-buds are treated with 1 part of HCl + 10 parts of alcohol for 5—8 minutes (for *Beta* and some other plants). The HCl-alcohol mixture can only be used one day.

(3) *Washing.* — Replace the loosening fluid with distilled water (cooled) and preserve the preparation in a cool state (8—12° C.; not above 15° C.). Washing is completed within 15—30 minutes. For the preparation of thick leaf-tissues and body complexes 60 minutes should give a more reliable result.

(4) *Staining.* — Place 4—5 roots on an object-glass. In daylight or light from a daylight lamp against a black background the zones undergoing brisk division in the root-tip can be distinctly seen. Cut away portions not showing divisions. Suck off the water with filter-paper. Dry leaves and bodies by laying them out on a filter-paper, then place them on the object-glass. As a leaf-specimen take a *small* piece from the base of a leaf on a briskly growing part of the stem. Squeeze flower-buds into pieces, whereupon rather large portions of the bracts are picked off with a pincette. One drop of staining solution (for preparation, see below) is added. Put the tissues into the fluid and place a cover-glass loosely over. Lay the object-glass on a thick, smooth plate of glass. The subsequent procedure is as follows: with an ordinary piece of stiff eraser rubber (*a*) tap gently on the cover-glass to ensure the fluid making contact with the tissue, then (*b*) press fairly gently over the whole cover-glass for short periods so that the fluid is pressed outwards to the edges. When the white tissues that have now been squeezed a little out are clearly visible, press (*c*) hard with short vibratory movements directly above the root-tips, though not so that the cover-glass slides. Now (*d*) lift the cover-glass cautiously by one edge, though only so much that the staining solution flows in over the tissues. Considerably less pressure is used for leaves; the divisions in the parenchyma are to be disclosed. Pollen mother-cells are pressed still more mildly. For roots the staining time is 1—2 minutes. The staining fluid should be cool, about 15° C. During the period of staining the preparation tolerates normal room temperature (16—18° C.), but must not be heated with a strong lamp or similar source of heat. Leaves of *Pisum* are stained for 30 seconds, those of *Pyrus* and *Betula* for 45—60 seconds. For these types of tissue *4 % staining fluid* is used. Buds, on the other hand, require staining for 4—5 minutes in a 3 % solution. When staining pollen mother-cells care should be taken not to lift the cover-glass as the large pollen mother-cells may thereby be damaged. The pigment colours the chromosomes quite black and the plasm a faint grey.

When the preparation has been stained, lay a filter-paper over the preparation glass and pass a rubber-roller to and from some ten times over

the cover-glass, preferably also crosswise. Over the root-tips roll at first gently, then rather hard; on leaves rather gently the whole time and on the pollen mother-cells very gently. A good preparation should not be vesicular and the tissue ought to be squeezed out to at least fivefold its size.

The preparation is then ready for immediate microscopical determination. In cool temperature it will keep for 24—36 hours, leaf preparations for 2—3 days. For work in green light it is suggested that a white dull-glass filter is to be placed before the lamp and a thin green glass in the iris diaphragm of the microscope. The chromosomes now will show up quite black and the plasm colourless, but some of the luminous brightness is thereby lost.

(5) *Staining fluid.* — Prepare a 4 % solution as follows: dilute 50 c. cm. of concentrated (98 %) acetic acid with distilled water to 100 c. cm., so that the fluid contains about 50 % of acetic acid. Heat this solution to boiling-point. Add, continuously stirring the while, 4 gms. of *spirit-soluble nigrosine* (see Stain-Technology, vol. 10, p. 73, 1935). The black staining fluid is to boil for 3—5 minutes, i. e. so long that it acquires a weak tarry consistence. When large batches are prepared, careful allowance should be made for the evaporative affect of the larger boiling volume, the boiling intensity, and so on. On removing the solution from the source of heat allow it to cool a few minutes at room temperature. Then cool it rapidly to room temperature and filter it immediately, taking care to avoid unnecessary evaporation. Keep the solution in a closed glass vessel in the dark under room temperature for about 10—14 days before use.

The above-described method is now being used at Hilleshög in actual practice. A girl gets the preparation ready, another one carries out the microscopical work; both were previously quite unacquainted with cytological and similar technique. After two or three days training their capacity was 60 plant-counts on root-tips per working day of seven hours. To-day this number is increased to 100—120. Leaf-counts are performed quicker than root-counts (150—200 slides per day).

Finally, it may be mentioned that the writer has conducted tests in *Abies* and *Pinus*. Counts were made without difficulty even in these genera, which are considered to offer difficulties for cytological work.

Beet Breeding Institute of the Swedish Sugar Company at Hilleshög, Landskrona. March, 1947.

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ARNE LUNDQVIST: On self-sterility and inbreeding effect in tetraploid rye.

The present investigation was started in the spring of 1945. Isolations (parchment baggings) were made both on diploid and tetraploid »Stålråg», a well-known commercial variety, from which a tetraploid strain was raised by Professor A. MÜNTZING more than five years ago.

As regards the self-sterility, this has turned out to be uneffaced in this material of tetraploid rye, but a weakening of the incompatibility is noticed, especially if the category with the lowest self-fertility (0—25 %) is taken into account.

TABLE 1. *Percentage of seed setting after selfing.*
(F_1 = isolations giving rise to a I_1 generation.)

		0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	100	n	$\overline{X}$
2n	*a* F_1 1945	47																		47	2,50
2n	*b* F_1 1946	140	5	1	1		1		1	3		5	6	4	5	7	3			182	13,98
2n	*c* F_1 1942	160	8	1	1	1			1			1								173	3,48
4n	*d* F_1 1945	65	11	3	2															81	3,92
4n	*e* F_1 1946	158	12	8	4	3	1	1												187	4,18
2n	*f* I_1 1946	52	1	2								1	1	3	1					61	8,16
4n	*g* I_1 1946	94	14	3																111	3,40

The high average degree of self-fertility in the *b* series is caused by the great number of individuals with percentages ranging from 50 to 80. Their occurrence is probably due to the fact that the material of kernels from which the *b* series was raised was obtained from plants in standard plots in a large material of »Stålråg» that had inbred for about 20 generations and had reached a high degree of self-fertility. Thus, the progeny of this standard material must have been infested with factors promoting self-fertility. For the sake of comparison data reported by MÜNTZING (1943) are included in the *c* series.

There is a close agreement in the lower classes of fertility between the

a, *b* and *c* series. The *a* and *d* series were compared; a χ^2 test applied to the classes 0—5—100 gave a χ^2 of 10,624 and a P slightly larger than 0,001. The same comparison between the *c* and *e* series applied to the classes 0—5—10—100 gave a χ^2 of 6,840 and a P intermediate between 0,05 and 0,02. This difference in self-fertility is the more significant as the fertility after open pollination is lower in the tetraploids (about 80 % in the diploids, 60 % in the tetraploids).

The data of the I_1 series *f* and *g* also indicate a weakening of the incompatibility in the tetraploids. The occurrence of individuals in classes of high fertility in the diploid series, however, results in an average more than twice the value of that of the tetraploids.

Regarding the influence of inbreeding, both the diploids and tetraploids show inbreeding degeneration, but the effect, though quite considerable, is almost in each case less marked in the tetraploids. Regarded separately, the differences are frequently not highly significant, but the significance is to be found in the agreement of the results. Lack of space does not permit a report of all results from comparisons in various characters, but in order to demonstrate the general tendency the data from the two characters showing the greatest difference between diploids and tetraploids in inbreeding effect after one generation of selfing are given below.

TABLE 2. *Plant height in cm. Diploids and tetraploids.*

	40	50	60	70	80	90	100	110	120	130	140	150	160	n	$\bar{X}$	m
2n Pop. 1946				1			12	21	37	52	27	4		154	129,42	1,05
2n I_1 1946			3	3	7	12	15	13	8	4	2			67	105,30	2,27
4n Pop. 1946			1		1	5	13	17	56	47	13	2		155	125,71	1,09
4n I_1 1946	1	1	1	1	6	3	16	22	30	11	1			93	114,25	1,84

TABLE 3. *Average number of spikelets per ear. Diploids and tetraploids.*

	0	5	10	15	20	25	30	35	40	45	n	$\bar{X}$	m
2n Pop. 1946				1	8	20	71	49	5		154	33,15	0,37
2n I_1 1946		2	1	3	8	16	31	5	1		67	28,92	0,81
4n Pop. 1946			2	4	8	31	77	31	2		155	31,47	0,41
4n I_1 1946		2	3	2	8	21	47	10			93	29,54	0,65

I hope soon to be able to treat these questions more in detail and to present all the results. As a rather limited first I_1 generation has been the base of this investigation of the effect of inbreeding (25 lines in the diploids, 32 in the tetraploids), the experiments will be repeated on a larger scale and extended in various ways.

Institute of Genetics, Lund, Sweden, February 22nd, 1947.

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NILS NYBOM: Accessory chromosomes in *Allium*.

In a small sample of *Allium Porrum* plants the present writer found an inflorescence the pollen grains of which contained a varying number of ac-

cessory chromosome elements, at least of two different sizes. Fig. 1 shows a pollen grain with one long (not yet identified) and five short accessory chromosomes in addition to the 16 normal ones.

Preliminary studies show that these accessory chromosomes undergo a normal disjunctional division at the pollen mitosis. Thus, they have not the ability of the B-chromosomes of *Secale* and *Anthoxanthum* to increase their number by preferential non-disjunction at the pollen mitosis.

Accessory chromosomes are known from a great number of plants and animals. However, the question of their properties and function has acquired a new significance through the contributions of MÜNTZING (e. g. 1946) and

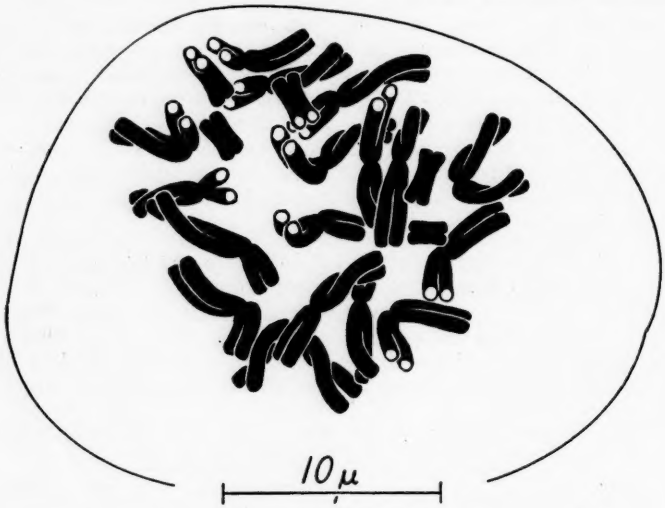

Fig. 1. A pollen grain with accessory chromosomes.

ÖSTERGREN (1945, 1947). As a part of the rather extensive works on B-chromosomes that are carried out and designed at the Institute of Genetics, Lund, the present writer intends to perform a cytological investigation of the seed material originating from the *Allium* plants mentioned.

Lund, Institute of Genetics, February, 1947.

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TYGE W. BÖCHER: Cytological studies of *Arabis Holboellii* HORNEM.

In a list of chromosome numbers of arctic and boreal plants (BÖCHER, 1938) the haploid number $n = 22$ for *Arabis Holboellii* was mentioned. This count was based upon investigations of a large number of metaphase plates of the first pollen mitosis. Later, a number of counts were made by ROLLINS (1941) in pollen mother-cells of *Arabis Holboellii* var. *retrofracta* and var. *pinetorum*. According to ROLLINS, var. *retrofracta* has $n = 7$ and 14, and var. *pinetorum* $n = 14$ and 21. As pointed out by ROLLINS, the number $n = 22$ is not compatible with the numbers found by him, and this fact makes more cytogenetic work of this species necessary.

In order to clear up some of the problems I have collected and studied more material of the species. This material, as well as that studied earlier, was from the Botanical Gardens in Copenhagen. The plants cultivated here are undoubtedly descendants of plants from West Greenland. They belong to *Arabis Holboellii* var. *typica*, which occurs in Greenland and in northern North America.

In the first pollen mitosis 22 chromosomes, or perhaps more correctly $21 +$ a very small extra chromosome, were counted again. But the same number could further be established in root-tip mitosis, in some few embryo-sac cells, and in some cells in embryos. In the metaphases in the PMC's a varying number of bivalents, trivalents, and univalents are present. In several cases, however, complete asynapsis occurred. In most pollen-sacs both meiotic divisions were omitted. The PMC's form a kind of restitution nucleus with $21 + 1$ chromosomes. Thus, the pollen-grains with $21 + 1$ chromosomes are monade-pollen and originate from unreduced nuclei containing the somatic chromosome number. In some of the pollen-sacs studied, however, dyads are also produced, resulting in smaller pollen-grains which frequently degenerate.

The material strongly supports the idea that *Arabis Holboellii* var. *typica* is a triploid type containing an extra chromosome of small size. Furthermore, apomixis is very probable. Some castration experiments show that pollination is necessary for seed production. Hence the plant may be pseudogamic.

A more detailed paper on the subject is planned and may appear when studies on some new material collected in 1946 in different localities in West Greenland have been completed.

Plant-Anatomical Institute, University of Copenhagen, March, 1947.

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ÅKE GUSTAFSSON: The advantageous effect of deleterious mutations.

Lethal mutations are common in cross-fertilizing populations of plants and animals. Owing to their recessive character they do not immediately show

up in nature, but they readily become unmasked in laboratory experiments. Often such mutations consist of short deficiencies, inactivating or destroying part of the chromosome substance. They are generally regarded as loss mutations, draw-backs in evolution, having no positive value whatever.

In 1946 the author argued that this latter conclusion did not fit the facts. On the contrary, many lethals increase the individual viability and plasticity in heterozygous condition, although they destroy the organism in homozygous state. Lethal genes are not merely negative; some of them act as valuable tools in the dynamics of the species.

Exact model experiments are difficult to carry through in cross-fertilizing organisms, even in *Drosophila*. They require homozygosity of most or all genes, except the deleterious one. Viability and competivity must be precisely and adequately measured. Fertilization by controlled gametes ought to take place. An agricultural plant like barley immediately fulfils these requirements. In the pure line Golden barley, isolated in 1897, several spontaneous chlorophyll mutations have been obtained. Two of them, *albina* 7 and *xantha* 3, have been repeatedly used in experiments (GUSTAFSSON, 1946). The monohybrids of these lethals show a distinct superiority to their normal sister plants, although the differences require a large material in order to be definitely proved. An obvious feature is, however, their longer preservation of seed germination.

Some years ago these two mutations were combined to form a dihybrid. They segregate independently of each other. No hybrid sterility is present. All plants of a large dihybrid offspring were analysed in 1946—1947 with regard to the number of spikes per plant, number of kernels per plant and kernel weight per plant. Afterwards the genotype was determined for every plant in the offspring. The results are shown in Table 1.

Previous analyses of monohybrid offsprings indicated a perspicuous though small heterosis effect. This becomes strikingly increased in the dihybrid condition. The two deleterious mutations add their effects. Spike

TABLE 1. *The increased viability of chlorophyll heterozygotes in a dihybrid offspring.*

	No. of spikes per plant	Prop.	No. of kernels per plant	Prop.	Kernel weight per plant (gram)	Prop.
Dihybrid (*AaBb*)	$5{,}47 \pm 0{,}17$	*1,19*	$114{,}16 \pm 3{,}82$	*1,18*	$4{,}54 \pm 0{,}16$	*1,15*
A7-monohybrid (*AaBB*)	5,05	1,10	104,76	1,08	4,17	1,06
X3-monohybrid (*AABb*)	4,88	1,07	104,66	1,08	4,20	1,06
Normal type (*AABB*)	$4{,}58 \pm 0{,}22$	1	$96{,}62 \pm 4{,}91$	1	$3{,}95 \pm 0{,}23$	1
Diff. dihybrid/normal type	$P = 0{,}001$		$0{,}01 > P > 0{,}001$		$0{,}05 > P > 0{,}02$	

number is heightened by 19 %, kernel number by 18 % and kernel weight — the property of special agricultural value — by 15 %. The differences between dihybrid and normal type (Golden barley) are statistically significant. The increase in total kernel weight becomes even more remarkable, taking into account that 38 % of the seeds of the dihybrid plants are recessive for one of the mutations and 6 % for both of them.

JONES (1945) published important data on monohybrid heterosis in inbred lines of maize. These lines have no immediate value as yield varieties. Golden barley, on the other hand, was for several years the best two-row barley in North Europe and is still locally cultivated. All the best Scandinavian cross varieties (Kenia, Maja, Balder, Ymer) are partially built up on its genotype. After thirty years of continuous work the barley yield has been increased by about thirteen or fourteen per cent above the level of Golden barley. What such prominent plant-breeders as HANS TEDIN, VESTERGAARD, NILSSON-EHLE did not fully accomplish — the fifteen per cent increase —, is here in principle achieved by two deleterious mutations.

A further analysis shows that dihybrids and monohybrids are specially apt to profit by favourable environmental conditions. More readily than their normal sister plants they produce a high spike and kernel number, as well as a high kernel weight. They increase the range of variation, augment the plasticity (Table 2). Also here the monohybrids lie midway between the dihybrid and the normal state.

TABLE 2. *The increased variation in positive direction of dihybrid and monohybrid chlorophyll mutations.*

	No of spikes per plant			No. of kernels per plant			Kernel weight per plant (gram)			No. of plants
	>7	>8	>9	>180	>210	>240	>7	>9	>11	
	% of all plants			% of all plants			% of all plants			
Dihybrid	17,6	13,3	8,2	12,9	6,9	3,9	13,3	5,6	1,3	233
Monohybrids (average) ...	11,3	6,0	3,5	7,0	2,5	2,1	10,2	3,2	0,4	284
Normal type	8,6	2,5	0,0	6,2	2,5	0,0	8,6	2,5	0,0	81

In consequence, a genotype with half its offspring having lethal character can be conspicuously superior to its normal relatives. And — the fundamental fact — this superiority is effected by the deleterious mutations themselves. This advantageous effect probably holds true of numerous lethals in natural populations of plants and animals, man not to be forgotten.

Svalöf, Institute of Genetics. April, 1947.

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TILL MEDARBETARNA.

Manuskript — helst *maskinskrivna* — torde insändas till Redaktionen (Adelgatan 3, Lund) i fullt tryckfärdigt skick. De böra vara *noga genomsedda* för undvikande av ändringar mot manuskriptet. Obs. kommateringen! Korrektionskostnaderna betalas av författaren. Korrektur ställes till Redaktionen. Direkt förbindelse mellan författaren och tryckeriet tillåtes icke.

Personnamn sättas med KAPITÄLER. De markeras i manuskriptet med en våglinje. Latinska namn på växter och djur samt ord och satser av särskild vikt *kursiveras* (enkel understrykning).

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Planscher numreras med romerska siffror och de i dem ingående bilderna med arabiska.

Tabeller åsättas arabiska siffror och förses med kort rubrik.

Citerade arbeten samlas i en litteraturförteckning. I texten hänvisas till denna genom angivande av författare och årtal. Har en författare utgivit flera publikationer under samma år, tilläggas efter årtalet små bokstäver (a, b, c, etc.). Samma beteckningssätt användes i litteraturlistan, vilken uppställes i alfabetisk ordning efter författarna och under dessa i kronologisk följd. Inga litteraturhänvisningar få göras genom fotnoter. Överhuvudtaget så få noter som möjligt!

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INNEHÅLL.

Sid.

SUOMALAINEN, ESKO, Parthenogenese und Polyploidie bei Rüsselkäfern (Curculionidae). (With a Summary in English.) 425
LEVAN, ALBERT, Studies on the camphor reaction of yeast 457
STEINEGGER, ERNST and LEVAN, ALBERT, The cytological effect of chloroform and colchicine on *Allium* 515
HÅKANSSON, ARTUR, Some observations on the seed development in Ecuadorian cacao 526
LIMA-DE-FARIA, A., Disturbances in microspore cytology of *Anthoxanthum* 539
LEVAN, ALBERT and STEINEGGER, ERNST, The resistance of *Colchicum* and *Bulbocodium* to the c-mitotic action of colchicine 552

ABSTRACTS — KURZE MITTEILUNGEN.

VON ROSEN, GÖSTA, The rapid nigrosine-method for chromosome counts, applicable to all the growing tissues of the plant 567
LUNDQVIST, ARNE, On self-sterility and inbreeding effect in tetraploid rye 570
NYBOM, NILS, Accessory chromosomes in *Allium* 571
BÖCHER, TYGE W., Cytological studies of *Arabis Holboellii* HORNEM. 573
GUSTAFSSON, ÅKE, The advantageous effect of deleterious mutations 573

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